UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OCT 10 1996

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of Mutagenicity, Developmental, Oral, and Inhalation Toxicity Studies conducted with Omacide® (3-Iodo-2-propynyl butylcarbamate).

TO: Walter Francis / Karen Leavy
Product Manager # 31
Registration Division (7505C)

FROM: Timothy F. McMahon, Ph.D. 8/7/96
Pharmacologist, Review Section I
Toxicology Branch II, Health Effects Division (7509C)

THRU: Yiannakis M. Ioannou, Ph.D. Y M. Ioannou 8/8/96
Section Head, Review Section I
Toxicology Branch II, Health Effects Division (7509C)

and

Stephanie R. Irene, Ph.D. Yiannakis M. Ioannou for 8/8/96
Acting Chief, Toxicology Branch II
Health Effects Division (7509C)

Registrant: Olin Corporation

Action Requested: Review of Mutagenicity studies; review of Developmental Toxicity Studies in the Rat and Rabbit; review of Inhalation Toxicity Studies (5-Day, 2-Week, and 13-Week) in the rat; review of a subchronic oral toxicity study in the rat.

(1)

EPA Identification Numbers:

MRID numbers: 43491803; 43491804; 43491805; 43491813; 43530203; 43530204; 43530205; 43530206; 43530207;43530213.

P.C. Code: 107801

DP Barcodes: D212151; D212153; D212154; D212156; D212158; D212159; D212160; D212163; D212164; D212165

Submission: S481260

Recommendations/Conclusions

Toxicology Branch II has reviewed the submitted Toxicology data in support of registration of Omacide® IPBC for use in adhesives, caulks, patching compounds and grouts, metalworking fluids and concentrates, paints and stains, wood preservation, plastics, and plastic coatings, paper coatings, textiles, canvas, and cordage, and inks. The data are considered acceptable and will support registration of Omacide® IPBC. However, it is recommended that the uses for Omacide® be limited to those previously granted to Troy Corporation for Troysan Polyphase IPBC, based on toxicological concerns of dermal irritation and cholinesterase inhibition after repeated exposure.

The Health Effects Division Reference Dose / Peer Review Committee (RfD) met on September 12, 1996 to consider the data submitted in support of the registration of Omacide® IPBC. The committee recommended that an RfD not be assigned to this chemical, due to the lack of a complete database for RfD assessment, and the classification of Omacide® IPBC as a non-food use chemical. Minor changes were recommended to the data evaluation records for the 90-day toxicity study in rats and the developmental toxicity study in rabbits. These changes have been incorporated into the data evaluation records. In addition, the committee recommended that an acute neurotoxicity study in rats (§81-7) with cholinesterase activity monitoring and measurement be conducted to address the concern of neurotoxic effects as a result of the observed cholinesterase inhibition in the subchronic inhalation study with Omacide.

Executive Summaries

1) MRID #'s 43491803 (range finding); 43530204 (definitive): Twomey, K. (1994) Omacide (IPBC): Oral (gavage) Rat Developmental Toxicity (Teratogenicity) Study. Toxicol Laboratories Limited, Ledbury, Herefordshire, England. Study Nos. OLA/18/R (range-finding) and OLA/19/R. August, 1994.

In a developmental toxicity study (MRID 43530204), omacide (>97% ai) was administered to 24 female CD (Sprague-Dawley) rats per dose by gavage at dose levels of 0, 25, 75, or 250 mg/kg/day from days 6 through 15 of gestation.

Maternal toxicity as evidenced by aggressive behavior, post-dose salivation, decreased mean body weight gain, and decreased food consumption was observed in the mid-dose (75 mg/kg/day) and high-dose (250 mg/kg/day) rats. During the dosing period (days 6-15), the mean body weight gain of high- and mid-dose dams were approximately 75 and 83%, respectively of the controls. At the onset of dosing (days 6-9 of gestation), food consumption by the high-dose group was 71% of the controls ($p < 0.01$) and remained lower (not significant) throughout the dosing period. In the mid-dose group, food consumption was approximately 84% of the controls ($p < 0.01$) from days 6 to 12 of gestation. Thereafter, mean food consumption was similar to the controls. Additionally at necropsy, absolute and relative liver weights were approximately 17-22% greater ($p < 0.01$) in the high-dose dams as compared to the controls. **The maternal LOEL is 75 mg/kg/day, based on decreased mean body weight gain and food consumption. The maternal NOEL is 25 mg/kg/day.**

No evidence of a treatment-related effect on fetal viability was demonstrated. At 250 mg/kg/day, mean fetal body weight was reduced to approximately 95-96% of the controls (significant in the females at $p < 0.05$) and developmental delays that included a higher frequency of rib defects and incomplete or non-ossification of bones were noted.

The developmental LOEL is 250 mg/kg/day based on reduced body weight and developmental delays. The developmental NOEL is 75 mg/kg/day.

The developmental toxicity study in the rat is classified **acceptable** and does satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3 (a) in rats.

2) MRID #'s 43491804 (range-finding) and 43530205 (definitive): Twomey, K. (1994) Omacide (IPBC): Oral (gavage) Rabbit Developmental Toxicity Study. Toxicol Laboratories Limited, Ledbury, Herefordshire, England. Study Nos. OLA/20/R (range-finding) and OLA/26/R. August, 1994.

In a developmental toxicity study (MRID 43530205), omacide (>97% ai) was administered to 16-18 female New Zealand White rabbits per dose by gavage at dose levels of 0, 10, 20, or 40 mg/kg/day from days 7 through 19 of gestation.

Maternal toxicity was evidenced by marked deterioration in condition resulting in the premature sacrifice (days 15-22 of gestation) of one mid-dose and four high-dose females. The prematurely sacrificed animals had exhibited reduced body weight gain and food consumption from the start of dosing. Although food consumption during days 7-19 was decreased similarly at all dose levels (26-30%), food efficiency was not significantly affected during this period. Additionally at the final necropsy, absolute and relative liver weights of the high-dose does were 7-10% greater than the concurrent controls. **The maternal LOEL is 40 mg/kg/day, based on clinical signs of toxicity. The maternal NOEL is 20 mg/kg/day.**

In the 40 mg/kg/day dose group, there was a decrease in number of total live fetuses and live fetuses/dam (6.7 compared to 8.5-8.7 for other groups) that was accompanied by a decrease in implantations/dam (7.8 compared to 9.2 for controls). Post-implantation loss was also increased at the 40 mg/kg/day dose level vs control. The decrease in total implantations may be based partly on the increase in preimplantation loss (which occurs before dosing and is not compound related). Sex ratio (% male) was higher than the concurrent and historical controls, and the observed visceral and skeletal changes were noted mainly in one fetus in one litter at 10 mg/kg/day or 40 mg/kg/day. There were no effects on pregnancy outcome, gravid uterus weights, nor any teratogenic findings. **Based on the decreased total live fetuses, live fetuses/dam, and increased post-implantation loss, the developmental LOEL is determined to be 40 mg/kg/day. The Developmental NOEL = 20 mg/kg/day.**

The developmental toxicity study in the rabbit is classified **acceptable** and does satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3b) in rabbits.

3) MRID # 43491813: Kenny, T.J. 1994. Omacide IPBC. 5-Day Repeat Dose Inhalation Toxicity Study in Rats. Huntingdon Research Centre Ltd., Huntingdon PE18 6ES, England, Laboratory Project Number TXC 8/942212. November 9, 1994.

In an inhalation toxicity study (MRID 43491813), omacide (3-iodo-2-propynl butylcarbamate; >97% ai) was administered to 5 rats/sex/dose by whole-body exposure at nominal concentrations of 0, 0.4, 1.0, 4.0 mg/m^3 (measured concentrations of 0, 0.3, 1.0 and 3.8 mg/m^3, respectively; 0, 0.0003, 0.001, or 0.0038 mg/L, respectively) for 6 hours per day on 5 consecutive days.

Male and female rats in the 4.0 mg/m^3 dose group had histopathologic lesions of the larynx (epithelial hyperplasia in the ventral region and hyperplasia or squamous metaplasia in the ventrolateral regions, with necrosis of the underlying cartilage); in addition, males exhibited a slight reduced body weight gain. Male and female rats in the 0.001 mg/L dose group had the same larynx lesions reported in the 4.0 mg/m^3 dose rats. No adverse effects were seen in the 0.4 mg/m^3 exposure level rats. **The LOEL is 0.001 mg/L (1.0 mg/m^3), based on the presence histopathologic lesions of the larynx. The NOEL is 0.0003 mg/L (0.3 mg/m^3).**

This toxicity study provided **supplemental** data, but was not conducted according to Subdivision F guidelines. It can be used as a pilot range-finding study.

4) MRID # 43530213: Kenny, T.J. (1994) Omacide IPBC. 2-Week repeat dose inhalation toxicity study in rats. Huntingdon Research Center Ltd., Huntingdon, Cambridgeshire, PE18 6ES, England. Laboratory Project Number TXC 6/932373. December 8, 1994.

In an inhalation toxicity study (MRID 43530213), omacide (3-iodo-2-propyl-butylcarbamate; >97% ai) was administered to 5 rats/sex/dose by whole-body exposure at nominal concentrations of 0, 12, 40, or 80 mg/m^3 (actual concentrations 0, 4, 10, 38 and 67 mg/m^3, respectively; 0, 0.004, 0.01, 0.038 and 0.067 mg/L, respectively) for 6 hours per day. Rats in the 0, 4, and 12 mg/m^3 treatment groups were exposed 5 days per week for 2 consecutive weeks; exposure of rats in the 40 or 80 mg/m^3 groups was terminated after 3 days because of the severity of the toxic reactions.

In the 80 mg/m^3 dose group, mortality occurred (4/10), both sexes exhibited clinical signs of toxicity during exposure (agitated grooming of snout, half or fully closed eyes, licking inside of mouth, gasping and rubbing chin on

the grid mesh floor) and after exposure (noisy respiration; sneezing; gasping; brown staining around snout, jaws and forepaws; red ears; red limbs; and discharges from the snout/nostrils). There were marked bodyweight losses, and reduced food and water consumption. Rats that died exhibited high incidences of lung congestion, and all rats in this group had gaseous distention and minimal contents of the gastrointestinal tract. In the 40 mg/m^3 dose group, mortality occurred (one female), both sexes exhibited clinical signs of toxicity during exposure and after exposure that were similar to those at the higher dose. There was significantly reduced bodyweight gain, and reduced food and water consumption in males. For humane reasons, all surviving rats in the 80 and 40 mg/m^3 dose groups were sacrificed after the third exposure. In the group exposed at 12 mg/m^3, agitated grooming, half closed eyes, noisy respiration, and brown staining around the snout and jaws were observed. Weight gain was decreased significantly in both sexes. After 2 weeks of exposure, males had increased liver weights; both sexes exhibited high incidences of gaseous distention and minimal contents of the cecum; and both sexes exhibited histolopathologic lesions of the respiratory system (epithelial hyperplasia of the ventral region of the larynx, squamous metaplasia in the ventrolateral region of the larynx accompanied by necrosis of the underlying cartilage). In the group exposed at 4 mg/m^3, both sexes exhibited the same histopathologic lesions described above, but clinical signs were absent. **The LOEL is 4.0 mg/m^3, based on the occurrence of histopathologic lesions of the larynx. A NOEL was not established.**

This toxicity study provides **supplemental** data, but was not conducted according to Subdivision F guidelines. It can be used as a pilot range-finding study.

5) MRID # 43530203: Kenny, T.J. (1994) Omacide IPBC. 13-Week Inhalation Toxicity Study in Rats. Huntingdon Research Center Ltd., Huntingdon, Cambridgeshire, PE18 6ES, England. Laboratory Project Number TXC 7/942772. November 3, 1994.

In a subchronic inhalation toxicity study (MRID 43530203), Omacide (>97% ai) was administered to Sprague-Dawley rats (15 rats/sex/dose) by whole-body exposure at nominal concentrations of 0, 0.25, 1.25, or 6.25 mg/m^3 (measured concentrations of 0, 0.0003, 0.00116, and 0.0067 mg/L, respectively; or 0, 0.3, 1.16, and 6.7 mg/m^3) for 6 hours per day, 5 days per week, for 13 consecutive weeks. The 0.25 mg/m^3 dose group was repeated based on exposure of the original 0.25 mg/m^3 dose group to twice the nominal concentration during weeks 6-8 of the study.

In the 6.25 mg/m^3 treatment group, plasma cholinesterase (ChE) activity was significantly reduced (approximately 20%) in males during weeks 2 and 13, and

erythrocyte ChE was decreased in females at study week 2. Brain cholinesterase activity was significantly reduced in males (17%) and females (25%), and hyperplasia or squamous metaplasia with necrosis of the ventral cartilage of the larynx was seen after 13 weeks of treatment. In the 1.25 mg/m^3 treatment group, brain cholinesterase levels were statistically significantly reduced in most of the females (25%; $p<0.05$). No effects were observed in the repeat 0.25 mg/m^3 treatment group. **The LOEL for systemic toxicity is 0.00116 mg/L (1.25 mg/m^3), based on depressed brain cholinesterase activity in females. The NOEL was 0.00023 mg/L (0.25 mg/m^3).**

This subchronic toxicity study is **acceptable** and satisfies the guideline requirement for a subchronic inhalation study (82-4) in rats.

6) Twomey, K. (1994) Omacide (IPBC) 13 Week Oral (gavage) Toxicity Study in the Rat. Toxicol Laboratories Limited, Herefordshire, England. Laboratory Project ID OLA/24/C. August 16, 1994.

In a subchronic toxicity study (MRID 43530202), Omacide (IPBC; 97-98% ai) was administered to 15 albino rats/sex/dose by gavage at dose levels of 25, 75, or 200 mg/kg/day for 13 weeks.

All treatment groups exhibited excess post-dose salivation; the frequency and severity were concentration-related. Abnormalities in liver function and/or pathology were also observed in all treatment groups. Blood cholinesterase was decreased by 11% in female rats at 75 mg/kg/day and by 17% at 200 mg/kg/day; similar decreases were not observed in the male treatment groups. Two males in the 25 mg/kg/day group exhibited dark livers. Increased incidence of abnormal shape of liver was observed in male rats at all dose levels, and in female rats at 75 and 200 mg/kg/day. Centrilobular hepatocyte hypertrophy was observed in 1/15 males in the 25 mg/kg/day treatment group; 4/15 males in the 75 mg/kg/day treatment group; and 15/15 males and 3/15 females in the 200 mg/kg/day group. Absolute liver weight in the 75 mg/kg/day females was increased 13% vs. controls, and at 200 mg/kg/day, liver weight was increased 29-32% vs. control in male and female rats. Increased relative liver weights were also observed in both sexes at 75 mg/kg/day (12-17% higher) and 200 mg/kg/day (39-41% higher) vs. controls. Also, increased relative kidney weights were noted in the 75 mg/kg/day males and in both sexes from the 200 mg/kg/day groups; no associated macroscopic or microscopic alterations were observed. Hyperkeratosis and squamous epithelial hyperplasia of the nonglandular region of the stomach were observed in all treatment groups, and stomach ulceration and chronic inflammation were observed in the male and female 200 mg/kg/day treatment groups. This effect was most likely due to the irritancy of the test substance. No treatment-related changes were observed in the clinical appearance, body weight, food consumption or ophthalmology parameters for any of the treatment groups. **The LOEL is 25 mg/kg/day for males and females, based on excessive post-dose salivation in both sexes,**

abnormal shape of livers in males, and squamous epithelial hyperplasia of the nonglandular region of the stomach in both sexes. The NOEL is <25 mg/kg/day for both sexes.

This subchronic toxicity study is graded as **acceptable**, and satisfies the guideline requirement for a subchronic oral toxicity study (§82-1a) in rats.

7) San, R.H.C. and Klug, M.L. (1993). Salmonella/Mammalian Microsome Plate Incorporation Assay (Ames Test) and Escherichia coli WP2 uvrA Reverse Mutation Assay With a Confirmatory Assay; Microbiological Associates, Inc., Rockville, MD; Study No. TC727.501088; Study Completion Date: March 31, 1993. MRID # 43530207.

In a microbial mutagenicity assay (MRID No. 43530207), Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 were exposed to 3.3-1000 µg/plate Omacide IPBC (99%) in the presence and absence of S9 activation and E. coli strain WP2 uvrA was exposed to 10-3333 µg/plate +/- S9. Two independent trials were conducted. The S9 fraction was derived from Aroclor 1254 induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

Cytotoxicity for all strains was observed at 1000 µg/plate +/- S9. The nonactivated and S9-activated positive controls induced the expected mutagenic response in the corresponding tester strain. **There was, however, no indication of a mutagenic effect at noncytotoxic doses of Omacide IPBC.** This study is classified as **acceptable.**

7) Bigger, C.A.H. and Clarke, J.J. (1993). CHO/HGPRT Mutation Assay With Confirmation; Microbiological Associates, Inc., Rockville, MD; Study No. TC727.332001; Study Completion Date: September 1, 1993. MRID No. 43491805.

In an <u>in vitro</u> mammalian cell gene mutation study (MRID # 43491805), Chinese hamster ovary (CHO) cells were exposed to Omacide IPBC (97.5%) doses of 5-20 µg.ml without S9 activation or 15-100 µg/ml with S9 activation in the initial trial. Concentrations evaluated in the confirmatory trial ranged from 2.5-20 µg/ml -S9 or 15-50 µg/ml +S9. The S9 fraction was derived from Aroclor 1254 induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

Cytotoxicity was apparent at ≥ 15 µg/ml -S9 and at ≥ 50 µg/ml +S9. The nonactivated and S9-activated positive controls induced the expected mutagenic response. **There was, however, no indication that Omacide IPBC induced a mutagenic effect.** This study is classified as **acceptable.**

8) Putman, D.L. and Young, R.R. (1993). Micronucleus Cytogenetic Assay in Mice; Microbiological Associates, Inc., Bethesda/ Rockville, MD; Study No. TC727.122; Study Completion Date: May 10, 1993. Unpublished. MRID No. 43530206.

In a mouse micronucleus assay (MRID # 43530206), groups of five male and five female ICR mice received single intraperitoneal injections of 28, 55, or 110 mg/kg Omacide IPBC (97.5%) prepared in corn oil. Animals were sacrificed at 24, 48, or 72 hours postexposure and bone marrow cells were examined for the incidence of micronucleated polychromatic erythrocytes (MPEs).

Overt toxicity in high-dose animals included death and lethargy. There was no evidence of a cytotoxic effect on the target tissue. The positive control induced the expected high yield of MPEs in both sexes. **There was, however, no evidence that Omacide IPBC induced a clastogenic or aneugenic effect.** This study is classified as **acceptable.**

012072

DATA EVALUATION RECORD

OMACIDE

Study Type: 82-4; 13-Week Inhalation Toxicity Study in Rats.

Work Assignment No. 1-8C (MRID 43530203)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer: Mike Norvell, Ph.D.	Signature:	Mike Norvell
	Date:	3/12/96
Secondary Reviewer William McLellan, Ph.D.	Signature:	W. L. McLellan
	Date:	3/12/96
Project Manager William Spangler, Ph.D.	Signature:	William J. Spangler
	Date:	03/12/96
Quality Assurance: Reto Engler, Ph.D.	Signature:	Reto Engler
	Date:	3/12/96

Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: T. McMahon, Ph.D. _____, Date 8/1/96
Review Section I, Toxicology Branch II (7509C)
EPA Secondary Reviewer: Y. Ioannou, Ph.D. _____, Date 8/14/96
Review Section I, Toxicology Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Inhalation Toxicity - Rat
OPPTS Number: 870.3465 OPP Guideline Number: §82-4

DP BARCODE: D212163 SUBMISSION CODE: S481260
P.C. CODE: 107801 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Omacide (>97% ai, IPBC)

SYNONYMS: 3-iodo-2-propynl butylcarbamate

CITATION: Kenny, T.J. (1994) Omacide IPBC. 13-Week Inhalation Toxicity Study in Rats. Huntingdon Research Center Ltd., Huntingdon, Cambridgeshire, PE18 6ES, England. Laboratory Project Number TXC 7/942772. November 3, 1994. MRID 43530203. Unpublished.

SPONSOR: Olin Corporation, 91 Shelton Avenue, PO Box 30-9643, New Haven, CT 06511

EXECUTIVE SUMMARY:

In a subchronic inhalation toxicity study (MRID 43530203), Omacide (>97% ai) was administered to Sprague-Dawley rats (15 rats/sex/dose) by whole-body exposure at nominal concentrations of 0, 0.25, 1.25, or 6.25 mg/m^3 (measured concentrations of 0, 0.0003, 0.00116, and 0.0067 mg/L, respectively; or 0, 0.3, 1.16, and 6.7 mg/m^3) for 6 hours per day, 5 days per week, for 13 consecutive weeks. The 0.25 mg/m^3 dose group was repeated based on exposure of the original 0.25 mg/m^3 dose group to twice the nominal concentration during weeks 6-8 of the study.

In the 6.25 mg/m^3 treatment group, plasma cholinesterase (ChE) activity was significantly reduced (approximately 20%) in males during weeks 2 and 13, and erythrocyte ChE was decreased in females at study week 2. Brain cholinesterase activity was significantly reduced in males (17%) and females (25%), and hyperplasia or squamous metaplasia with necrosis of the ventral cartilage of the larynx was seen after 13 weeks of treatment. In the 1.25 mg/m^3 treatment group, brain cholinesterase levels were statistically significantly reduced in most of the females (25%; $p<0.05$). No effects were observed in the repeat 0.25 mg/m^3 treatment group. **The LOEL for systemic toxicity is 0.00116 mg/L (1.25 mg/m^3), based on depressed brain cholinesterase activity in females. The NOEL was 0.00023 mg/L (0.25 mg/m^3).**

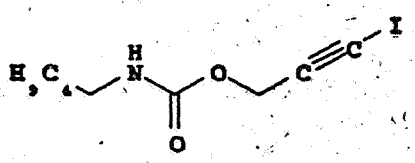
This subchronic toxicity study is acceptable and satisfies the guideline requirement for a subchronic inhalation study (82-4) in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality statements were provided. Flagging statements were not provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Omacide IPBC
   Description: White powder
   Lot/Batch #: 2DR-293-TSI
   Purity: >97% ai
   Stability of compound: Expiration date of sample reported to be September 1994
   CAS #: Not provided
   Structure:



2. Vehicle and/or positive control: None

3. Test animals: Species: Rat
   Strain: Sprague Dawley Crl:CD BR
   Age and weight at study initiation: Approximately 6-7 weeks of age; males 241-334 g, females 170-239 g
   Source: Charles River (UK) Limited, Manston Road, Kent, England
   Housing: Housed in groups of 5 in suspended stainless steel cages with mesh tops and bottoms. Exposures took place in the same room. After the start of the exposure period, each group was positioned on an individual cage battery. Each battery was placed in a separate ventilated cabinet in the holding area to avoid the possibility of inhalation of test substance from the fur of rats in other groups.
   Diet: SDS Rat and Mouse #1 Modified Maintenance Diet (Special diet services, Witham Essex, UK), ad libitum
   Water: Municipal tap water, ad libitum
   Environmental conditions:
   Temperature: 15.5-23.5 C
   Relative Humidity: 29-60% (deviations below 40% occurred on 4 separate occasions as follows: 29% for approximately

5 hr.; 36% for approximately 6 hr.; 30% for approximately 17 hr.; 25% for approximately 2 hr.).
Air changes: Data not provided
Photoperiod: 12-hour light/dark cycle
Acclimation period: 2 weeks

B. STUDY DESIGN

1. In life dates - Initial Start: 12/13/93 End: 5/16/94
Repeat of low dose Start: 2/16/94 End: 5/31/94

2. Animal assignment

Animals were weighed and assigned by a computer program to the test groups to obtain approximately equalized group mean body weights. Table 1 summarizes the concentrations (nominal, measured), the mass median aerodynamic diameter (MMAD), geometric standard deviation (GSA), and number of rats in each treatment group.

TABLE 1: STUDY DESIGN.[a]

Target Concentration (mg/m^3)	Measured Concentration (mg/m^3)	Rats per sex[b]	Particle size[c] (%)	
			<6μm	<3.5μm
0	0	15	--	--
0.25[d]	0.30 ± 0.11	15	67.5	52.4
0.25[d]	0.23 ± 0.04	15	75.4	60.7
1.25	1.16 ± 0.28	15	78.0	64.7
6.25	6.69 ± 2.25	15	75.4	59.5

[a] Data obtained from pages 26-28 of the study report
[b] Five animals/group were identified as a satellite group for cholinesterase determinations.
[c] Because the aerosol did not contain a normal distribution of particle sizes (one population had a MMAD of 1.55-3.5 micrometers and one a MMAD of less than 0.5 micrometers), size distributions of this type cannot be described by a single MMAD.
[d] Because the rats originally exposed to omacide at 0.25 mg/m^3 were found to have, in fact, been exposed at twice that rate during weeks 6-8, the low dose portion of the study was repeated.

3. Generation of the test atmosphere and description of the chamber

A Wright dust generator was used to suspend dust in a stream of dry air. The dust-laden air was passed through a narrow bore jet into an exhaust nozzle with a baffle and into an elutriator/expansion chamber, where it was mixed with air before entering the exposure chamber. Exposure chambers (stainless steel and glass) with an internal volume of approximately 0.75 m^3 were fitted with flow meters and six sampling ports. A 3-inch tubular perforated exhaust plenum was situated at the bottom of the chamber and connected to a drainage system.

Wright dust feed operating conditions data are found in Appendix 3, pages 91 and 92 of the report, and individual sample values of test article chamber atmosphere concentrations are found in Appendix 5, pages 96-117 of the report.

4. Time to equilibrium

The time to equilibrium was not clearly stated in the report, but duration of test material was 60 minutes with a system air flow of 150 L/minute per exposure period.

5. Analytical Chemistry

The test material was analyzed by HPLC (Appendix 2, pages 45-46 of the report)

Test atmosphere concentration - The chamber nominal concentrations were calculated from the amount of test substance over the exposure periods and the total air flow through the chambers during those periods. At a minimum of approximately 1, 3, and 5 hours during each 6-hour exposure period (twice for the control exposures), samples of test atmosphere were withdrawn at 10 L/minute through a glass fiber filter supported in an open-faced filter holder. Filters were weighed before and after sampling and then extracted with appropriate solvents for subsequent chemical analysis. Results are summarized in Table 1 (above).

Particle size determination - Chamber air was withdrawn through a Marple 296 cascade impactor at a rate of 2 L/minute. Various impactor stages were used to fractionate the aerosol particulates according to their average aerodynamic equivalent particle diameter. The deposited test article was washed off each stage and analyzed by HPLC according to methods described in Appendix 2 (page 46) of the report. Data in Table 2 (pages 43-46 of the report) indicate that the test aerosols did not contain a normal distribution of particle sizes. At least two populations of aerosols were generated, one having a median

aerodynamic diameter of between approximately 1.55 and 3.5 μm and the other with a median aerodynamic diameter of less than 0.5 μm. For this reason, the particle size determination data are summarized as percent of particles with a size less than 6 and 3.5 μm diameter (Table 1; above). The study authors estimated that an "approximate 50% median value of 3 μm was applicable to all groups on study."

6. Statistics

For all parameters, the analyses were carried out separately for males and females. Bodyweight data were analyzed using weight gains, and body weight, food consumption, clinical pathology, and organ weight data were statistically analyzed using Bartlett's Test for homogeneity of variance between treatments unless the data consisted predominantly of one particular value. In that case, the proportion of animals with values different from the mode was analyzed by other appropriate methods. If significant heterogeneity was found, a logarithmic transformation was tried to see if a more stable variance structure could be obtained. If no significant heterogeneity was found (or if a satisfactory transformation was found), a one-way analysis of variance was carried out. If significant heterogeneity of variance was detected, and could not be removed by a transformation, the Kruskal-Wallis analysis of ranks was used. Analysis of variance was followed by Student's 't' and William's Tests for dose related responses, and Kruskal-Wallis analyses were followed by Shirley's Test for non-parametric data. Where appropriate, analysis of covariance was substituted for AOV and for organ weights, and final bodyweights were used as a covariance to account for those bodyweights that have affected the organ weights. At the discretion of the pathologist, Fisher's Exact Test was used for certain incidences of histopathological findings. The reviewer has no objections to the statistical procedures used in evaluating the data generated in this study.

C. METHODS:

1. Observations

All animals were inspected at least twice a day for signs of toxicity and mortality. During exposures, clinical signs were recorded either as a group response (all visible animals responding similarly) or as an individual response (one particular rat affected).

2. Body weight

Each rat was weighed weekly, beginning 1 week before the start of exposures and continuing through the end of the study.

3. Food consumption

Food consumption for each cage of animals was recorded weekly, starting one week before the start of exposures until the end of the study. Mean daily diet consumption was calculated as gm food/kg body weight/day.

4. Ophthalmoscopic examination

The eyes of all rats were examined once prior to the start of the exposures. The eyes of all Main group rats were also examined once during week 13 of study. Eyes of examined rats were treated with drops of 0.5% tropicaimide solution prior to examination.

5. Blood

Blood was collected from all Main group rats via the orbital sinus using light anesthesia with ether after an overnight fast, during week 13 of study. Blood samples were taken from all Satellite group rats during study weeks 2 and 13, immediately after exposures from 60 animals for hematology and clinical analysis. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)	X	Mean corpusc. volume (MCV)
X	Platelet count	X	Reticulocyte count
	Blood clotting measurements	X	Packed cell volume
X	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
X	Phosphorus	X	Total Cholesterol
X	Potassium	X	Globulins
X	Sodium	X	Glucose
		X	Total bilirubin
		X	Total serum protein (TP)
	ENZYMES		Triglycerides
			Serum protein electrophores
X	Alkaline phosphatase (ALK)		
X	Cholinesterase (ChE)		
X	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase (also ALT, SGPT)		
X	Serum aspartate aminotransferase (also AST, SGOT)		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

6. Urinalysis

Urinalysis was not performed in this study.

7. Sacrifice and Pathology

All animals that were sacrificed on schedule were subject to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

17

	DIGESTIVE SYSTEM
X	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
XX	Liver*+
	Gall bladder*
X	Pancreas*

	RESPIRATORY
X	Trachea*
XX	Lung*
X	Nose
X	Pharynx
X	Larynx

	CARDIOVASC./HEMAT.
X	Aorta*
X	Heart*
X	Bone marrow*
X	Lymph nodes*
X	Spleen*
X	Thymus*

	UROGENITAL
XX	Kidneys*+
X	Urinary bladder*
XX	Testes*+
XX	Epididymides
X	Prostate
X	Seminal vesicle
X	Ovaries*+
X	Uterus*
	Vagina
	Ureter

	NEUROLOGIC
XX	Brain*+
X	Periph.nerve*
X	Spinal cord (3 levels)*
X	Pituitary*
X	Eyes (optic n.)*

	GLANDULAR
X	Adrenal gland*
X	Lacrimal gland
X	Mammary gland
X	Parathyroids*++
X	Thyroids*++

	OTHER
X	Bone*
X	Skeletal muscle*
X	Skin*
X	All gross lesions and masses*

* Required for subchronic toxicity studies.
+ Organ weight required in subchronic studies.
++ Organ weight required for non-rodent studies.

Following terminal sacrifice, brains of satellite animals were removed, cut in half, and a half brain from each rat weighed and deep frozen in dry ice/hexane. Brain ChE was determined in the frozen half by the method of Ellman et al.

II. RESULTS:

A. Observations

1. Mortality - No unscheduled deaths were reported.

2. Clinical Signs - Red ears, which were considered to be a non-specific response to a mild irritant, were seen in the 6.25 mg/m^3 treatment group following exposure on exposure days 3, 4 and 5. Closed/half-closed eyes were also seen in high dose rats for the first three exposures only. No other treatment-related signs of toxicity were reported in this study.

B. Body weight and weight gain

There were no treatment-related effects on body weight or body weight gain reported for this study. Mean body weight gain

values (g/rat at study weeks 0-4, 4-8, and 8-13) generated in this study are summarized in Table 2.

TABLE 2. MEAN BODY WEIGHT GAINS (G) OF RATS SUBJECTED TO INHALATION EXPOSURES TO OMACIDE.[a]

Exposure (mg/m^3)	Weeks of Exposure					
	0-4	5-8	9-13	0-4	5-8	9-13
	Males			Females		
0	123	89	61	61	36	20
0.30[b]	103	74	55	62	35	20
0.23[b]	133	90	55	59	33	25
1.16	116	88	61	59	34	21
6.69	108	90	55	59	33	25

[a] Calculated by reviewer using data obtained from Table 5, pages 52-53, in the study report.
[b] Because the rats originally exposed to omacide at 0.25 mg/m^3 were found to have, in fact, been exposed at twice that rate during weeks 6-8, the low dose portion of the study was repeated.

C. Food consumption

There were no treatment-related effects on food consumption reported for this study.

D. Ophthalmoscopic examination

There were no treatment-related ophthalmoscopic findings reported for this study.

E. Blood work

1. Hematology - After 13 weeks of exposures, males in all dose groups except the repeated 0.25 mg/m^3 dose group had slight (2.8%), but statistically significantly depressions in mean corpuscular volume. Females in the 6.25 mg/m^3 dose group had statistically significant changes in mean corpuscular hemoglobin concentration (2.3% depressed), reticulocytes (47% depressed), hematocrit (4.1% increased), and erythrocyte count (4.4% increased). None of these changes was considered of toxicologic importance.

2. Clinical chemistry - With the exception of males in the 6.25 mg/m^3 treatment group, males and females in all dose groups had significantly ($p < 0.05$) higher (males 10.7-17.5%,

females 20.7-39.1%) serum GPT levels. Females in the 6.25 mg/m^3 dose group had significantly lower (12.5%) serum albumin levels. Serum glucose levels in the 1.25 and 6.25 mg/m^3 dose females were also significantly increased.

After 13 weeks of treatment, the 6.25 mg/m^3 dose males had depressed mean plasma (26%) and brain (16.8%) cholinesterase levels, and the 6.25 mg/m^3 dose females had depressed mean erythrocyte (39%) and brain (26%) cholinesterase levels (Tables 3 and 4). In the 1.25 mg/m^3 dose group, females also had 24.6% depressed brain cholinesterase levels. All these values were statistically significant.

TABLE 3. CHOLINESTERASE ACTIVITY IN MALES AFTER 2 AND 13 WEEKS OF TREATMENT.[ab]

Weeks of Exposure	Exposure Level (mg/m^3)			
	0	0.25/0.25[c]	1.25	6.25
Plasma Cholinesterase Activity (µmol/mL/minute)				
2	0.46	0.46/0.40	0.40	0.36**
13	0.44	0.42/0.42	0.44	0.36*
RBC Cholinesterase Activity (µmol/mL/minute)				
2	1.41	1.37/2.83	1.52	1.59
13	1.68	1.74/1.37	1.54	1.07
Brain Cholinesterase Activity (µmol/mL/minute)				
13	5.77	5.02/7.17	5.05	4.80*

[a] Data obtained from Table 9, pages 62-69, in the study report.
[b] Historical data (page 31 of text): plasma cholinesterase 0.47 ± 0.21 µmol/mL/minute, RBC cholinesterase 2.03 ± 0.37 µmol/mL/minute, brain cholinesterase 6.31 ± 1.209 µmol/mL/minute.
[c] Because the rats originally exposed to omacide at 0.25 mg/m^3 were found to have, in fact, been exposed at twice that rate during weeks 6-8, the low dose portion of the study was repeated.
* Statistically different from control, $p < 0.05$.
** Statistically different from control, $p < 0.01$.

20

TABLE 4. CHOLINESTERASE ACTIVITY IN FEMALES AFTER 2 AND 13 WEEKS OF TREATMENT.[ab]

Weeks of Exposure	Exposure Level (mg/m^3)			
	0	0.25/0.25[c]	1.25	6.25
Plasma Cholinesterase Activity (μmol/mL/minute)				
2	0.83	0.79/1.01	0.88	0.73
13	1.27	1.46/1.36	1.28	1.29
RBC Cholinesterase Activity (μmol/mL/minute)				
2	1.46	1.46/1.67	1.47	0.99*
13	1.90	1.59/1.22	2.03	2.00
Brain Cholinesterase Activity (μmol/mL/minute)				
13	6.59	6.38/7.21	4.97*	4.87*

[a] Data obtained from Table 9, paged 62-69, in the study report.
[b] Historical data (page 31 of text): plasma cholinesterase 0.47 ± 0.21 μmol/mL/minute, RBC cholinesterase 2.03 ± 0.37 μmol/mL/minute, brain cholinesterase 6.31 ± 1.209 μmol/mL/minute.
[c] Because the rats originally exposed to omacide at 0.25 mg/m^3 were found to have, in fact, been exposed at twice that rate during weeks 6-8, the low dose portion of the study was repeated.
* Statistically different from control, $p < 0.05$.

F. Urinalysis

Urine was not collected during this study.

G. Sacrifice and pathology

1. Organ weight - There were no treatment-related effects on organ weights seen in this study.

2. Gross pathology - There were no treatment-related gross pathological effects seen in this study.

3. Microscopic pathology

a) Non-neoplastic - All rats in the 6.25 mg/m^3 dose group (6.7 mg/m^3) had epithelial hyperplasia in the ventral region of the larynx and necrosis in the ventral

cartilage of the larynx; epithelial hyperplasia of the larynx over the arytenoids (15/15 males and 5/15 females); epithelial ulceration in the ventral region of the larynx (4/15 males and 1/15 females); atrophy of the submucosal glands of the larynx (3/15 males and 6/15 females); and aggregates of macrophages in the lungs (7/15 males, 2/15 females).

Aggregates of macrophages in the lungs were also seen in 3/15 males and 3/15 females in the 1.25 mg/m^3 dose group (1.16 mg/m^3); 4/15 males and 2/15 females in the 0.25 mg/m^3 dose group (0.3 mg/m^3); none in the repeat 0.25 mg/m^3 dose group; and 4/15 males and 2/15 females in the control group. Rats in the original 0.25 mg/m^3 dose group also had epithelial hyperplasia in the ventral region of the larynx (4/13 males and 7/13 females) and necrosis in the ventral cartilage (2/13 males and 3/13 females). The replacement group exposed to the 0.25 mg/m^3 dose level showed no abnormalities of the larynx. One 0.05 mg/m^3 dose female had epithelial hyperplasia over the arytenoids.

b) Neoplastic - All rats in the 6.25 mg/m^3 dose group had squamous metaplasia in the ventral region of the larynx.

III. DISCUSSION

A. Investigator's Conclusions

The investigator proposes a NOEL of 0.23 mg/m^3 for this study based on an absence of treatment-related toxicological findings in all rats in this repeat low dose group (Group 5). Significant treatment-related toxicological findings were seen in rats in the 1.16 mg/m^3 dose rats (Group 3) and above.

B. Reviewer's Discussion

The reviewer concurs with the investigator's interpretation of the data and conclusion.

IV. STUDY DEFICIENCIES

This subchronic toxicity study is acceptable and satisfies the guideline requirement for a subchronic inhalation study in the rat. Minor deficiencies in the study are:

The Time to Equilibrium (Item B4 above) could not be clearly stated.

During the study, there were problems with the dust generation system (aggregation and clogging) in the low and high exposure groups. For the low group, the range of concentrations was 0.14-0.53 mg/m^3; for exposures 1-6 the mean was 0.167 mg/m^3, and for exposures 29-37 (weeks 5-6), mean concentrations were 0.53 mg/m^3. At the high-exposure level, mean concentration at days 1-6 was 4.55 mg/m^3, at days 16-17 were 1.75 and 1.06 mg/m^3; between exposures 20-31, the mean concentration was 9.58 mg/m^3. The high excursions did not appear to be correlated with any adverse effects, but they are not acceptable. In addition, the MMAD could not be determined because there was a bimodal distribution of particle sizes. It was reported that the test laboratory made every effort to remedy the problem, including micronization of test material.

012072

DATA EVALUATION RECORD

OMACIDE

Study Type: 83-3b; Prenatal Developmental Study with Rangefinding - Rabbits

Work Assignment No. 1-8H/1-8I (MRIDs 43491804/43530205)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
Mary Menetrez, Ph.D.
Signature: Mary Menetrez
Date: 3/2/96

Secondary Reviewer
William McLellan, Ph.D.
Signature: W. L. McLellan
Date: 3/2/96

Project Manager
William Spangler, Ph.D.
Signature: William J Spangler
Date: 03/02/96

Quality Assurance:
Reto Engler, Ph.D.
Signature: Reto Engler
Date: 3-02/96

Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: T. McMahon, Ph.D. _____, Date 8/7/96
Review Section I, Toxicology Branch II (7509C)
EPA Secondary Reviewer: Y. Ioannou, Ph.D. _____, Date 8/8/96
Review Section I, Toxicology Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Study - Rabbit
OPPTS Number: 870.3700 OPP Guideline Number: §83-3b

DP BARCODE: D212159; D212160 SUBMISSION CODE: S481260
P.C. CODE: 107801 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Omacide (97-98 ai%, IPBC)

SYNONYMS: 3-iodo-2-propynl butylcarbamate

CITATION: Twomey, K. (1994) Omacide (IPBC): Oral (gavage) rabbit developmental toxicity study. Toxicol Laboratories Limited, Ledbury, Herefordshire, England. Study Nos. OLA/20/R (range-finding) and OLA/26/R. August, 1994. MRIDs 43491804 (range-finding) and 43530205. Unpublished.

SPONSOR: Olin Corporation, 91 Shelton Avenue, PO Box 30-9643, New Haven, CT 06511

EXECUTIVE SUMMARY:

In a developmental toxicity study (MRID 43530205), omacide (>97% ai) was administered to 16-18 female New Zealand White rabbits per dose by gavage at dose levels of 0, 10, 20, or 40 mg/kg/day from days 7 through 19 of gestation.

Maternal toxicity was evidenced by marked deterioration in condition resulting in the premature sacrifice (days 15-22 of gestation) of one mid-dose and four high-dose females. The prematurely sacrificed animals had exhibited reduced body weight gain and food consumption from the start of dosing. Although food consumption during days7-19 was decreased similarly at all dose levels (26-30%), food efficiency was not significantly affected during this period. Additionally at the final necropsy, absolute and relative liver weights of the high-dose does were 7-10% greater than the concurrent controls. **The maternal LOEL is 40 mg/kg/day, based on clinical signs of toxicity. The maternal NOEL is 20 mg/kg/day.**

In the 40 mg/kg/day dose group, there was a decrease in number of total live fetuses and live fetuses/dam (6.7 compared to 8.5-8.7 for other groups) that was accompanied by a decrease in implantations/dam (7.8 compared to 9.2 for controls). Post-implantation loss was also increased at the 40 mg/kg/day dose level vs control. The decrease in total implantations may be

based partly on the increase in preimplantation loss (which occurs before dosing and is not compound related). Sex ratio (% male) was higher than the concurrent and historical controls, and the observed visceral and skeletal changes were noted mainly in one fetus in one litter at 10 mg/kg/day or 40 mg/kg/day. There were no effects on pregnancy outcome, gravid uterus weights, nor any teratogenic findings. **Based on the decreased total live fetuses, live fetuses/dam, and increased post-implantation loss, the developmental LOEL is determined to be 40 mg/kg/day. The Developmental NOEL = 20 mg/kg/day.**

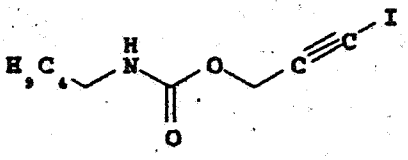
The developmental toxicity study in the rabbit is classified **acceptable** and does satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3b) in rabbits.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Omacide
   Description: Technical, off-white coarse powder, formulations in 1% aqueous methylcellulose were stable for 8 days at 4 C.
   Lot/Batch #: 2DR-293-TSI
   Purity: 97-98% ai
   CAS #: 55406-53-6
   Structure:



2. Vehicle: 1% aqueous methylcellulose

3. Test animals: Species: Rabbits
   Strain: New Zealand White
   Age at mating: 4 months
   Weight at mating: 3-4 kg
   Source: Interfauna U.K. Limited, Huntingdon, Cambridgeshire
   Housing: Grid-bottomed metal cages
   Diet: SQC Rabbit Standard, ad libitum
   Water: Tap water, ad libitum

Environmental conditions:
Temperature: 15-22 C
Humidity: 30-61%
Air changes: 16/hour
Photoperiod: 12 hours dark/12 hours light
Acclimation period: 5-6 days

B. PROCEDURES AND STUDY DESIGN

1. In life dates - Start: 11/29/93 End: 12/20/93

2. Mating:Mating occurred at the supplier's premises. For mating, each female was observed to copulate with one male and then given an injection of chorionic gonadotrophin. The day of mating was considered day 0 of pregnancy.

3. Animal Assignment: Animals were assigned to dose groups as indicated in Table 1. Assignments were based on body weight.

TABLE 1. ANIMAL ASSIGNMENT.

Test Group	Dose (mg/kg/day)	Number of Females
Control	0	16
Low (LDT)	10	16
Mid (MDT)	20	16
High (HDT)	40	18

4. Dose selection rationale

In a range-finding study (MRID 43491804), omacide was administered orally to groups of 5 female rabbits each at 0, 10, 25, 75, or 100 mg/kg/day in 1% aqueous methylcellulose. The test solutions were administered at a volume of 2 ml/kg on days 7 through 19 of gestation, inclusive. Individual doses were adjusted daily to body weight. Clinical observations, maternal body weight, and food consumption data were recorded. One female treated at 10 mg/kg/day died within 5 days of the start of dosing due apparently to poor dosing technique. Females found dead or sacrificed prematurely were necropsied immediately. On day 28 of gestation, the remaining females were sacrificed and gross necropsy was performed. The uteri and ovaries were examined to determine the status of each conceptus, including the number and distribution of implantations, resorptions, and live or dead fetuses. The liver and gravid uterus weights and the number of corpora lutea were also recorded. Live

fetuses were weighed, sexed, and examined for external abnormalities. The following were sacrificed and necropsied prematurely because of treatment-related reduced maternal body weight gain and food consumption: two females treated at 25 mg/kg/day (days 18 and 25 of pregnancy), three does treated at 75 mg/kg/day (days 18 of pregnancy), and all the females treated at 100 mg/kg/day (days 15 or 16 of pregnancy). The reduction in maternal body weight gain and decrease in food consumption was different ($p < 0.05$ or 0.01) from the controls in females dosed at 75 or 100 mg/kg/day. Abnormalities of the liver and gastrointestinal tract were observed at necropsy (premature or final sacrifice) of animals dosed at 25, 75, or 100 mg/kg/day.

There were no maternal effects observed in does dosed at 10 mg/kg/day. There were no dose related effects on fetal parameters.

Based upon the results of this range-finding study and an additional preliminary developmental toxicity study (not submitted), 40 mg/kg/day was selected as a high dose for the subsequent full developmental toxicity study in rabbits. Low- and mid-dose levels chosen were 10 and 20 mg/kg/day, respectively.

5. Dosage preparation and analysis

Test formulations were prepared twice by mixing appropriate amounts of the test substance with 1% aqueous methylcellulose and stored in the dark in brown glass bottles at 4 C. Prior to the start of the study, homogeneity was tested in samples taken from each of three levels (top, middle, and bottom) of test formulations prepared at low (1.0 mg/ml) and high (100 mg/ml) concentrations in 1% aqueous methylcellulose. Further samples were assayed after 1, 2, 4, and 8 days storage at 4 C to determine stability of the test substance. Concentration analyses of each test formulation were performed on the days of preparation.

Results

Homogeneity Analysis: 90-110%
Stability Analysis: 91-110%
Concentration Analysis: 90-101%

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

6. Dosage administration

All doses were administered once daily by gavage, on gestation days 7 through 19, in a volume of 2 ml/kg of body weight. Dosing was based on the most recent body weight determination.

C. OBSERVATIONS

1. Maternal Observations and Evaluations

From day 3 of gestation, all animals were checked daily for mortality or clinical signs. Body weights were recorded on days 0, 3 to 19, and on days 22, 25, and 28 of gestation. Food consumption data were recorded for days 3-7, every two days until day 27, and on day 28 of gestation. Six does were sacrificed prematurely (between days 15 and 27 of gestation), the other females were sacrificed on day 28 of gestation. Thoracic and abdominal cavities were opened, the major organs were examined, and macroscopic abnormalities were removed and fixed in formaldehyde. A urinalysis to detect blood proteins was performed on four of the females prematurely sacrificed. For females sacrificed on schedule the following were performed: the weight of the whole body, liver, and gravid uterus was recorded, and pregnancy status, number of corpora lutea, and number and distribution of implantation sites were noted. The implantations were classified as early or late resorptions, and dead or live fetuses.

2. Fetal Evaluations

Each fetus was removed and examined for external abnormalities. They were then killed by i.p. sodium pentobarbital. Each fetus was weughed then briefly fixed in alcohol. Later the same day, fetuses were skinned, dissected, the viscera examined, and sex determined by inspection of the internal genitalia. Fetuses were eviscerated prior to processing and staining with Alizarin red S for skeletal evaluation. Fetal findings were classified as variations or abnormalities (major or minor). All skeletal specimens were stored in aqueous glycerol with thymol to prevent fungal growth.

D. DATA ANALYSIS

1. Statistical analyses

Data were subjected to analysis of variance or Kruskal-Wallis test. Where significance was achieved, the data were subjected to Dunnett's t-test ($p<0.01$ or <0.05) or Dunn's multiple comparison test ($p<0.001$, <0.01, or <0.05).

2. Indices

Pre-implantation and post-implantation loss indices were calculated from cesarean section records of animals in the study. The pre-implantation loss (%) index was calculated as:

(# corpora lutea - # implantation sites)/# corpora lutea x 100

The post-implantation loss (%) index was calculated as:

(# implantation sites - # live fetuses)/# implantation sites x 100

3. Historical control data

Historical control data (February, 1990-May, 1993; 267 mated females) were provided to allow for comparisons with concurrent controls (Appendix 16, page 119 of the report).

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations

One mid-dose and four high-dose females were sacrificed during days 15 through 22 of gestation because of deteriorating condition. One control female aborted on day 27 of gestation; necropsy revealed perivaginal blood staining and hemorrhage of the left uterine horn. Reduced fecal output was observed in the mid-dose and high-dose animals. Red discharge around the vagina and red staining on the tray liner were observed in one control and one mid-dose female on days 19 and 23 of gestation. Brown perianal fur staining and gelatinous feces on the tray liner were observed in one low-dose female on day 27 of gestation.

2. Body Weight

Body weight gain data for animals that survived to day 28 and were pregnant are summarized in Table 2. The one female from the mid-dose group and the four females from the high-dose that were sacrificed prematurely had exhibited treatment-related body weight loss from the onset of dosing. At the start of dosing, the mean body weights of all treated groups were lower than the controls, the differences were significant ($p < 0.05$) in the low- and high-dose groups; the differences were attributed to the exclusion of non-pregnant females and females that had been sacrificed before day 28. During the dosing period (days 7-19), the mean body weight

gain for the three treatment groups was approximately 71-73% of the controls; this was attributed to the irritant nature of the test material. During the post-dosing period (days 19-28), the mean body weight gain for the three treatment groups was approximately 105-138% of the controls.

TABLE 2. MATERNAL BODY WEIGHT GAIN (KG).[a]

Interval	Dose in mg/kg/day (# of Does)			
	0 (12)	10 (13)	20 (12)	40 (12)
Pretreatment: Days 0-7	0.07 ± 0.11	0.06 ± 0.06	0.05 ± 0.08	0.06 ± 0.11
Treatment: Days 7-8	0.05 ± 0.03	0.00 ± 0.04*	0.02 ± 0.03	0.00 ± 0.06*
Treatment: Days 7-9	0.07 ± 0.05	0.06 ± 0.04	0.04 ± 0.05	0.04 ± 0.07
Treatment: Days 10-13	0.08 ± 0.05	0.07 ± 0.06	0.06 ± 0.10	0.07 ± 0.10
Treatment: Days 16-19	0.02 ± 0.06	0.03 ± 0.05	0.06 ± 0.14	0.04 ± 0.05
Treatment: Days 7-19	0.34 ± 0.08	0.25 ± 0.05	0.25 ± 0.14	0.24 ± 0.16
Posttreatment: Days 19-28	0.21 ± 0.06	0.22 ± 0.11	0.25 ± 0.10	0.29 ± 0.11

[a] Data obtained from Table 2, page 28, in the study report. Data on animals that were sacrificed prior to day 28 or were not pregnant were excluded by the authors.

* Significantly different from controls, $p < 0.05$.

3. Food Consumption

The five females sacrificed between days 15 and 22 of gestation had exhibited decreased food consumption from the onset of dosing. During days 3-7 (pretreatment interval), food consumption was 93-98% of the controls in all treated groups (excluding all prematurely sacrificed animals). Food consumption by the mid- and high-dose groups was reduced further during dosing (days 7-19) and was only approximately 73-76% of control ($p < 0.01$ or < 0.05) during days 11-15 of gestation. Although body weight gain was decreased by 26%, 26%, and 30% at the low, mid, and high dose respectively during the treatment period (days 7-19), food efficiency was not significantly affected (5.3, 4.1, 5.0, and 4.9 for te control, low dose, mid dose, and high dose, respectively).

Food consumption by the low-dose group was approximately 86% of the controls (nonsignificant) throughout the dosing period. During days 19-25 and 25-28 (post-treatment interval), food consumption was similar to the controls in the 10 and 20 mg/kg/day groups, but increased by 13% and 21% in the high-dose group in the two time periods, respectively.

4. Gross Pathology

Four high-dose females were sacrificed prior to the scheduled sacrifice on day 28 of gestation; at necropsy, liver mottling/pallor, reddened/ulcerated stomach mucosa, blood/blood proteins in the urine, and fluid intestinal tract contents were observed. At necropsy of the one mid-dose female prematurely sacrificed, blood/blood proteins in the urine as well as reddened stomach mucosa with raised white areas were observed. These findings were judged to be treatment-related. The liver weights of 3/4 of the high-dose animals sacrificed early were 20-30% lower than the mean weight in controls, but the liver-to-body weight ratios were similar to controls.

At the scheduled sacrifice (day 28 of gestation), the mean absolute and relative liver weights were 7-10% greater in the high-dose does as compared to the controls; these findings were not statistically significant, but were judged to be treatment-related. No treatment-related findings were detected in the low- or mid-dose animals.

5. Cesarean Section Data

Cesarean section data for pregnant females at the scheduled necropsy on day 28 of gestation are summarized in Table 3. The pregnancy rate was lower than normal, but was not different between the groups and it was within range of the historical means. In the high-dose does the following were observed: the corpora lutea/dam was 86% of the concurrent controls and was slightly below the range of historical control group means; there was a decrease in number of live fetuses/dam (78% of the controls) that was accompanied by a decrease in implantations/dam (85% of controls); The number of viable fetuses was within the range of historical control group means and the decrease in implantations may be partially contributed to by preimplantation loss (which occurs before dosing and not compound related). The sex ratio (% male) in the high-dose group was higher than the concurrent controls and slightly higher than the historical control range. There were no treatment-related effects observed in postimplantation loss or mean pup weights in the high-dose group.

No treatment-related findings were detected in the low- or mid-dose animals.

TABLE 3. CESAREAN SECTION OBSERVATIONS. [a]

Observation	Dose (mg/kg/day)			
	0	10	20	40
# Animals Assigned (Mated)	16	16	16	18
# Animals Pregnant	12	13	12	12
Pregnancy Rate (%)	(80)	(81)	(80)	(83)
# Nonpregnant	3	3	3	2
Maternal Wastage				
# Died (pregnant/nonpregnant)	0	0	0[b]	0[b]
# Aborted	1	0	0	0
# Premature Deliveries	0	0	0	0
Total # Corpora Lutea	122	136	127	106
Corpora Lutea/Dam	10.2	10.5	10.6	8.8
Total # Implantations	110	122	110	93
Implantations/Dam	9.2	9.4	9.2	7.8
Total # Litters	12	13	12	12
Total # Live Fetuses	103	110	104	80
Live Fetuses/Dam	8.6	8.5	8.7	6.7
Total # Dead Fetuses	0	0	0	0
Total # Resorptions[c]	7	12	6	13
Early	4	3	2	6
Late	3	9	4	7
Resorptions/Dam	0.58	0.92	0.50	1.08
Early	0.33	0.23	0.16	0.50
Late	0.25	0.69	0.33	0.58
Mean Fetal Weight (g) Males	37.0	34.8	37.3	38.3
Mean Fetal Weight (g) Females	36.2	34.3	36.5	34.3
Sex Ratio (% Male)	49	52	51	64
Preimplantation Loss (mn.litter%)	9.1	10.4	14.2	16.8
Postimplantation Loss (mn. litter%)	6.4	9.3	5.6	13.7

[a] Data were obtained from Tables 5 and 6, page 31-32, and Appendices 8 and 9, pages 66-73, in the study report.

[b] One female from mid-dose, and 4 females from high-dose groups were sacrificed prematurely due to deteriorating condition.

[c] No litters with total resorptions were reported.

B. DEVELOPMENTAL TOXICITY

Fetal examinations included external and visceral observations at necropsy and skeletal findings. The study report classified fetal findings as variations or malformations (major or minor) and provided a summary incidence of the number of fetuses and mean percent affected in each evaluation category. Noteworthy visceral and skeletal findings are summarized in Tables 4a and 4b.

1. External and Visceral Examination

There were 3 low-dose fetuses (3 litters) and 1 high-dose fetus with major abnormalities of the heart, brain, lungs, eyes, and head; the data are summarized in Table 4a. Minor abnormalities were detected in the controls and all treated groups and included two low-dose runted fetuses and one high-dose fetus with a kinked tail tip. Due to the low incidences and the lack of any apparent dose response, the external and visceral abnormalities and variants were not attributed to treatment with omacide.

2. Skeletal Examination

There were 3 low-dose (3 litters) and 2 high-dose (2 litters) fetuses with major skeletal abnormalities of the skull, vertebrae, and ribs; the findings are summarized in Table 4b. Minor abnormalities were detected in the controls and all treated groups and included incomplete ossification of the ribs, clubbed, bifurcated, or floating ribs, as well as incomplete ossification of the frontals, parietals, and interparietals. The total number of minor abnormalities were detected as follows; 17 control fetuses (10 litters), 26 low-dose fetuses (13 litters), 21 mid-dose fetuses (9 litters), and 15 high-dose fetuses (8 litters). Due to the low incidences and the lack of any apparent dose-response, the skeletal abnormalities and variants were not attributed to treatment with omacide.

TABLE 4a. EXTERNAL AND VISCERAL FETAL OBSERVATIONS AT NECROPSY.[a]

Observations	Dose (mg/kg/day)			
	0	10	20	40
#Fetuses(litters) examined	103 (12)	110 (13)	104 (12)	80 (12)
#Fetuses(litters) affected	0 (0)	3 (3)	0 (0)	1 (1)
Head: short	0 (0)	1 (1.1)	0 (0)	0 (0)
Frontal region: Short	0 (0)	1 (1.1)	0 (0)	0 (0)
Frontal region: Bulging	0 (0)	1 (1.1)	0 (0)	0 (0)
Jaw, upper: Cleft	0 (0)	0 (0)	0 (0)	1 (1.7)
Eye, uni- or bilateral: Microphthalmia	0 (0)	0 (0)	0 (0)	1 (1.7)
Encephaly	0 (0)	0 (0)	0 (0)	1 (1.7)
Hydrocephaly	0 (0)	1 (1.1)	0 (0)	0 (0)
Brain: No organized tissue structure	0 (0)	0 (0)	0 (0)	1 (1.7)
Brain: Reduced tissue	0 (0)	1 (1.1)	0 (0)	0 (0)
Nose: Short	0 (0)	0 (0)	0 (0)	1 (1.7)
Cleft palate	0 (0)	0 (0)	0 (0)	1 (1.7)
Mouth: Asymmetric opening	0 (0)	1 (1.0)	0 (0)	0 (0)
Interrupted aortic arch	0 (0)	1 (0.8)	0 (0)	0 (0)
Aortic arch: Constricted	0 (0)	1 (1.1)	0 (0)	0 (0)
Heart: Mis-shapen (in presence of other vessel abnormalities)	0 (0)	1 (0.8)	0 (0)	0 (0)
Lung, one or more lobes: Hypoplastic	0 (0)	1 (0.8)	0 (0)	0 (0)

[a] Number of fetuses (mean %); Mean %=sum of % of affected fetuses per litter/number of litters. Data obtained from Table 8, pages 34-35, in the study report. All abnormalities were categorized as major by study author.

35

TABLE 4B. FETAL SKELETAL EXAMINATIONS.[a]

Observations	Dose (mg/kg/day)			
	0	10	20	40
# Fetuses(litters) examined	103 (12)	110 (13)	104 (12)	80 (12)
# Fetuses(litters) affected	0 (0)	3 (3)	0 (0)	2 (2)
SKULL				
Parietal: Absent	0 (0)	0 (0)	0 (0)	1 (1.7)
Interparietal: Absent	0 (0)	0 (0)	0 (0)	1 (1.7)
Occipital: Absent	0 (0)	0 (0)	0 (0)	1 (1.7)
Palatine, median line of ossification: Absent	0 (0)	1 (1.1)	0 (0)	0 (0)
Incisors, upper: Absent	0 (0)	1 (1.1)	0 (0)	0 (0)
Premaxilla: Short	0 (0)	1 (1.1)	0 (0)	1 (1.7)
Nasal: Single bone	0 (0)	1 (1.1)	0 (0)	0 (0)
Frontal: Single bone	0 (0)	1 (1.1)	0 (0)	0 (0)
Premaxilla: Mis-shapen	0 (0)	0 (0)	0 (0)	1 (1.7)
Nasal: Mis-shapen	0 (0)	1 (1.1)	0 (0)	0 (0)
Frontal: Mis-shapen	0 (0)	1 (1.1)	0 (0)	1 (1.7)
Jugal: Malformed	0 (0)	1 (1.0)	0 (0)	0 (0)
Mandible: Malformed	0 (0)	1 (1.0)	0 (0)	0 (0)
Auditory malleus: Malformed	0 (0)	1 (1.0)	0 (0)	0 (0)
Palatine: Cleft	0 (0)	0 (0)	0 (0)	1 (1.7)
VERTEBRAE AND RIBS				
Scoliosis	0 (0)	1 (0.8)	0 (0)	0 (0)
One or more: Major fusion	0 (0)	1 (0.8)	0 (0)	1 (1.7)
STERNEBRA				
One or more: Major fusion	0 (0)	0 (0)	0 (0)	1 (1.2)

[a] Number of fetuses (mean %); Mean %=sum of % of affected fetuses per litter/number of litters. Data obtained from Table 7, page 33, and Appendix 9, pages 36-40, in the study report. All abnormalities were categorized as major by the study author.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS

The study report concluded that oral administration of omacide at 20 and 40 mg/kg/day to pregnant rabbits during organogenesis was associated with marked deterioration in condition resulting in the premature sacrifice (prior to 28 days of gestation) of four high-dose and one mid-dose female. 'The prematurely sacrificed animals had exhibited reduced body weight gain and food consumption from the start of dosing and at necropsy were found to have reddened/ulcerated stomach mucosa. The absolute and relative liver weights of the high-dose females sacrificed at 28 days of gestation were higher than the controls but this finding was not statistically significant. There was also an increase in pre- and post-implantations and decreased fetal weight in the high-dose females but no evidence of developmental adverse effects. Oral administration of omacide at 10 mg/kg/day produced minimal reductions in food consumption and body weight gain; these findings were judged to be related to the irritant nature of omacide upon administration. Dosing at 10 mg/kg/day produced no maternal adverse effects and dosing at 20 mg/kg/day produced no developmental adverse effects.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY

Following oral administration of the test substance, omacide (97% ai) to pregnant rabbits on days 7-19 of gestation, maternal toxicity was observed as evidenced by marked deterioration in condition resulting in the premature sacrifice of one mid-dose and four high-dose females. The prematurely sacrificed animals had exhibited reduced body weight gain and food consumption from the start of dosing. Food efficiency was unaffected during days 7-19 of the study (period of treatment). Additionally, the absolute and relative liver weights of the high-dose does sacrificed at 28 days gestation were higher relative to the controls.

Maternal LOEL = 40 mg/kg/day
Maternal NOEL = 20 mg/kg/day

2. DEVELOPMENTAL TOXICITY:

a. Cesarean Section Data: In the 40 mg/kg/day females there was a decrease in number of live fetuses/dam (6.7 compared to 8.5-8.7 for other groups) that was

accompanied by a decrease in implantations/dam (7.8 compared to 9.2 for controls). The decrease in total implantations may be partially contributed to by preimplantation loss (which occurs before dosing and not compound related). However, an increase in post-implantation loss was also observed at 40 mg/kg/day.

b. Altered Growth: No compound-related effects were observed.

c. Developmental Variations: There were major skeletal abnormalities of the skull, vertebrae, and ribs in the low-dose (10 mg/kg/day) and high-dose (40 mg/kg/day) fetuses. However, most of the changes were due to one fetus in one litter at 10 and 40 mg/kg/day each. Due to the low incidences and the lack of any apparent dose response, the skeletal abnormalities and variants were not attributed to treatment with omacide.

d. Malformations: A small number of abnormalities and variants of the heart, brain, lungs, eyes, and head were noted in the control and treated groups but were not attributed to treatment with omacide.

Based on the decreased total live fetuses, live fetuses/dam, and increased post-implantation loss, the developmental LOEL is determined to be 40 mg/kg/day. The Developmental NOEL = 20 mg/kg/day.

IV. STUDY DEFICIENCIES

The developmental toxicity study in the rabbit is classified acceptable and does satisfy the guideline requirement for a developmental toxicity study [OPPTS 870.3700; §83-3b] in rabbits.

C-012072

DATA EVALUATION RECORD

OMACIDE

Study Type: N/A; 2-Week Repeat Dose Inhalation Toxicity Study in Rats

Work Assignment No. 1-8B (MRID 43530213)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
Mike Norvell, Ph.D.

Signature: Mike Norvell
Date: 3/12/96

Secondary Reviewer
William McLellan, Ph.D.

Signature: W.L. McLellan
Date: 3/12/96

Project Manager
William Spangler, Ph.D.

Signature: William J. Spangler
Date: 3/12/96

Quality Assurance:
Reto Engler, Ph.D.

Signature: Reto Engler
Date: 3/12/96

Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: T. McMahon, Ph.D. _____, Date 8/1/96
Review Section I, Toxicology Branch II (7509C)
EPA Secondary Reviewer: Y. Ioannou, Ph.D. _____, Date 8/1/96
Review Section I, Toxicology Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Two-Week Inhalation Toxicity - Rat
OPPTS Number: None OPP Guideline Number: None

DP BARCODE: D212165 SUBMISSION CODE: S481260
P.C. CODE: 107801 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Omacide (97-98% ai)

SYNONYMS: IPBC; 3-iodo-2-propynl butylcarbamate

CITATION: Kenny, T.J. (1994) Omacide IPBC. 2-Week repeat dose inhalation toxicity study in rats. Huntingdon Research Center Ltd., Huntingdon, Cambridgeshire, PE18 6ES, England. Laboratory Project Number TXC 6/932373. December 8, 1994. MRID 43530213. Unpublished.

SPONSOR: Olin Corporation, 91 Shelton Avenue, PO Box 30-9643, New Haven, CT 06511

EXECUTIVE SUMMARY:

In an inhalation toxicity study (MRID 43530213), omacide (3-iodo-2-propyl-butylcarbamate; >97% ai) was administered to 5 rats/sex/dose by whole-body exposure at nominal concentrations of 0, 12, 40, or 80 mg/m^3 (actual concentrations 0, 4, 10, 38 and 67 mg/m^3, respectively; 0, 0.004, 0.01, 0.038 and 0.067 mg/L, respectively) for 6 hours per day. Rats in the 0, 4, and 12 mg/m^3 treatment groups were exposed 5 days per week for 2 consecutive weeks; exposure of rats in the 40 or 80 mg/m^3 groups was terminated after 3 days because of the severity of the toxic reactions.

In the 80 mg/m^3 dose group, mortality occurred (4/10), both sexes exhibited clinical signs of toxicity during exposure (agitated grooming of snout, half or fully closed eyes, licking inside of mouth, gasping and rubbing chin on the grid mesh floor) and after exposure (noisy respiration; sneezing; gasping; brown staining around snout, jaws and forepaws; red ears; red limbs; and discharges from the snout/nostrils). There was marked bodyweight losses, and reduced food and water consumption. Rats that died exhibited high incidences of lung congestion, and all rats in this group had gaseous distention and minimal contents of the gastrointestinal tract. In the 40 mg/m^3 dose group, mortality occurred (one female), both sexes exhibited clinical signs of

toxicity during exposure and after exposure that were similar to those at the higher dose. There was significantly reduced bodyweight gain, and reduced food and water consumption in males. For humane reasons, all surviving rats in the 80 and 40 mg/m^3 dose groups were sacrificed after the third exposure. In the group exposed at 12 mg/m^3, agitated grooming, half closed eyes, noisy respiration, and brown staining around the snout and jaws were observed. Weight gain was decreased significantly in both sexes. After 2 weeks of exposure, males had increased liver weights; both sexes exhibited high incidences of gaseous distention and minimal contents of the cecum; and both sexes exhibited histolopathologic lesions of the respiratory system (epithelial hyperplasia of the ventral region of the larynx, squamous metaplasia in the ventrolateral region of the larynx accompanied by necrosis of the underlying cartilage). In the group exposed at 4 mg/m^3, both sexes exhibited the same histopathologic lesions described above, but clinical signs were absent. **The LOEL is 4.0 mg/m^3, based on the occurrence of histopathologic lesions of the larynx. A NOEL was not established.**

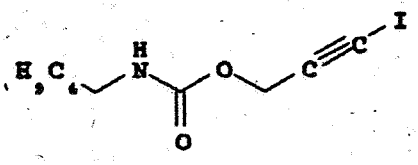
This toxicity study provides **supplemental** data, but was not conducted according to Subdivision F guidelines. It can be used as a pilot range-finding study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality statements were provided. Flagging statements were not provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Omacide
   Description: White powder
   Lot/Batch #: 2DR-293-TSI
   Purity: >97% ai
   Stability of compound: Expiration date of sample reported to be September 1994
   CAS #: Not provided
   Structure:



2. Vehicle and/or positive control: None

3. Test animals: Species: Rat

91

Strain: Sprague Dawley CD
Age and weight at study initiation: Approximately 6 weeks of age; males 277-388 g, females 181-222 g
Source: Charles River (UK) Limited, Manston Road, Kent, England
Housing: Housed in groups of five in suspended stainless steel cages with mesh tops and bottoms. Exposures took place in an adjacent room. After each exposure, each group was placed in a separate ventilated cabinet in a holding area to avoid cross contamination by test material that may have been dislodged from the fur.
Diet: SDS Rat and Mouse #1 SQC Modified Maintenance Diet, ad libitum (Special diet services, Witham Essex, UK)
Water: municipal tap water, ad libitum
Environmental conditions:
Temperature: 17.5-23.5 C
Relative Humidity: 33-60%
Air changes: Data not provided
Photoperiod: 12 hours light/dark cycle
Acclimation period: 1 day

B. STUDY DESIGN

1. In life dates - Start: 6/28/93 End: 7/12/93

2. Animal assignment

Animals were weighed and assigned to the test groups in Table 1 using a computer program to obtain approximately equalized group mean body weights at study initiation.

TABLE 1: STUDY DESIGN.[a]

Test group	Nominal Conc. (mg/m^3)	Measured Conc. (mg/m^3)	MMAD (μm)	GSA (μm)	Animals per sex
Control	0	0	--	--	5
Low	4	4	3.3	2.51	5
Low Mid.	12	10	2.5	2.15	5
High Mid.	40	38	2.4	1.99	5
High	80	67	2.4	1.97	5

[a] Data obtained from page 9 in the study report. An additional group of 5 rats/sex was retained as potential replacements for test group rats prior to the start of exposures. Reserve animals were removed from the study after the start of exposures.

3. Generation of the test atmosphere and description of the chamber

A Wright dust generator was used to suspend dust in a stream of dry air. The dust laden air was passed through a narrow bore jet into an exhaust nozzle with a baffle and into an elutriator/expansion chamber where it was mixed with air before entering the exposure chamber. Exposure chambers (stainless steel and glass) with an internal volume of approximately 0.5 m^3 were fitted with flow meters and six sampling ports. A 3-inch tubular perforated exhaust plenum was situated at the bottom of the chamber and connected to a drainage system.

4. Time to equilibrium

The time to equilibrium was not clearly stated in the study report.

5. Analytical Chemistry

The test material was analyzed by HPLC (Appendix 2, pages 56-57 of the report).

Test atmosphere concentration: The chamber nominal concentrations were calculated from the amount of test substance over the exposure periods and the total air flow

through the chambers during those periods. At a minimum of approximately 1, 3, and 5 hours during each 6-hour exposure period (twice for the control exposures), samples of test atmosphere were withdrawn at 10 L/minute through a glass fiber filter supported in an open-faced filter holder. Filters were weighed before and after sampling and then extracted with appropriate solvents for subsequent chemical analysis. Results are presented in Table 1 (above).

Particle size determination: Chamber air was withdrawn through a Marple 296 cascade impactor at a rate of 2 L/minute. Various impactor stages used to fractionate the aerosol particulates according to their average aerodynamic equivalent particle diameter. The deposited test article was washed off each stage and analyzed by HPLC according to methods described in Appendix 2 (page 46) of the report. Particle size data are summarized in Table 1 (above).

6. Statistics

The analyses were carried out separately for males and females using the individual animal as the experimental unit. Bodyweight data were analyzed using weight gains and organ weights were analyzed by ANOVA with final body weights as covariate. Fisher's Exact Test (for detecting general differences between differences) and Mantel's Test (for detecting dose-related trends) were utilized when the data consisted predominantly of one particular value (frequency of mode> 75%). Bartlett's test was used to test for homogeneity of variance between treatments. If significant heterogeneity was found, a logarithmic transformation was tried to see if a more stable variance structure could be obtained. If no significant heterogeneity was found (or if a satisfactory transformation was found), a one-way analysis of variance was carried out. If significant heterogeneity of variance was detected, and could not be removed by a transformation, the Kruskal-Wallis analysis of ranks was used. Student's 't' and William's tests for dose related responses followed ANOVA and the Kruskal-Wallis analyses were followed by Shirley's Test for non-parametric data.

C. METHODS

1. Observations

Animals were inspected twice daily for signs of toxicity and mortality. During exposure, clinical signs were recorded as a group response where all or a majority of animals appeared to be responding similarly. Responses were recorded individually where one particular rat was affected.

2. Body weight

Animals were weighed daily throughout the study, beginning one week prior to the first exposure.

3. Food consumption

Food consumption for each cage of animals was recorded daily and mean daily diet consumption was calculated.

4. Water Consumption

Water consumption in each cage of rats was recorded daily throughout the study, beginning one week before the first exposures.

5. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	X	Heart*	X	Periph.nerve*
	Esophagus*	X	Bone marrow*		Spinal cord (3 levels)*
	Stomach*	X	Lymph nodes*	X	Pituitary*
	Duodenum*	X	Spleen*	X	Eyes (optic n.)*
	Jejunum*	X	Thymus*		
	Ileum*				
	Cecum*		UROGENITAL		GLANDULAR
	Colon*	XX	Kidneys*+	X	Adrenal gland*
	Rectum*	X	Urinary bladder*		Lacrimal gland
XX	Liver*+	XX	Testes*+	X	Mammary gland
	Gall bladder*	XX	Epididymides	X	Parathyroids*++
X	Pancreas*	X	Prostate	X	Thyroids*++
		X	Seminal vesicle		
	RESPIRATORY		Ovaries*+		
X	Trachea*	X	Uterus*		OTHER
XX	Lung*		Vagina	X	Bone*
X	Nose				Skeletal muscle*
X	Pharynx				Skin*
X	Larynx				All gross lesions and masses*

* Required for subchronic toxicity studies.
+ Organ weight required in subchronic studies.
++ Organ weight required for non-rodent studies.

II. RESULTS:

A. Observations

1. Mortality - In the 80 mg/m^3 group, 2/5 males and 2/5 females were found dead after both exposures 2 and 3. In the 40 mg/m^3 group, 1/5 females were found dead after exposure 3. Congestion of the lungs (one female at 40 mg/m^3, 1/2 males and 2/2 females at 80 mg/m^3) was determined to be the cause of death. For humane reasons, all surviving rats in these dose groups were sacrificed after exposure 3.

2. Clinical Signs - At the 12 and 40 mg/m^3 exposure levels, both sexes exhibited agitated grooming of snout and half or fully closed eyes during exposures followed by noisy respiration; sneezing; gasping; brown staining around snout, jaws and forepaws; red ears; red limbs; and discharges from the snout/nostrils. In the 80 mg/m^3 group, in addition to the above symptoms, both sexes were observed licking the insides of the mouth, gasping, and rubbing of the chin on the grid mesh of

the cage floor. The incidence of observations (in particular gasping) was higher as dose increased.

There were no similar signs at the lowest exposure level (4 mg/m^3).

B. Body weight and weight gain

In the 80 and 40 mg/m^3 groups, both sexes exhibited marked decreases in bodyweight before they died or were sacrificed. Mean weights were depressed 21-24% for males and 10-12% for females when compared to controls. In the 12 mg/m^3 group, males exhibited statistically significantly reduced bodyweight gain. Two-week bodyweight gain data are summarized in Table 2.

Table 2. MEAN BODYWEIGHT GAINS AFTER 2 WEEKS OF EXPOSURE.[a]

Exposure Level (mg/m^3)	Males (g)	Females (g)
0	71	26
4	60	31
12	30*	27

[a] Data obtained from Table 5, page 43 in the study report.
* Significantly different from control, $p<0.05$.

C. Food consumption

In the 80 and 40 mg/m^3 dose groups, both sexes exhibited marked decreases in food consumption before they died or were sacrificed. In the 12 and 4 mg/m^3 dose groups, male rats exhibited reduced food consumption (29 and 13%, respectively). In females, a 16% decrease was observed at 12 mg/m3 and a 3% increase at 4 mg/m3.

D. Water Consumption

In the 80 and 40 mg/m^3 dose groups, both sexes exhibited marked decreases in water consumption before they died or were sacrificed. At the 12 and 4 mg/m3 dose levels, water consumption in males was reduced 15% and 13%, respectively. In females, water consumption was increased at 12 and 4 mg/m3 (10% and 19%, respectively).

E. Sacrifice and pathology

1. Organ weight - In the 12 mg/m^3 dose group, males exhibited statistically significantly increased absolute and relative (to

47

body and brain) liver weights. The mean liver weight (covariance adjusted for body weight) was 17% higher than in controls.

2. Gross pathology - Rats that died early or were sacrificed at the end of the study in the 80 and 40 mg/m^3 dose groups exhibited lung congestion, and gaseous distention and minimal contents of the gastrointestinal tract. Male rats in the 12 mg/m^3 dose group had minimal contents (2/5) and gaseous distention (5/5) of the caecum; females at this dose level exhibited no such symptoms.

3. Microscopic pathology

a) Non-neoplastic - In the 4 and 12 mg/m^3 dose groups, both sexes had epithelial hyperplasia of the ventral region of the larynx (3/5 males, 4/5 females in the 4 mg/m^3 group and 2/5 males and 4/5 females in the 12 mg/m^3 group), squamous metaplasia in the ventrolateral region of the larynx (3/5 males, 4/5 females in the 4 mg/m^3 dose group and 2/5 males and 4/5 females in the 12 mg/m^3 dose group) accompanied by necrosis of the underlying cartilage (4/5 males, 5/5 females in the 4 mg/m^3 dose group and 4/5 males and 5/5 females in the 12 mg/m^3 dose group). In the 4 mg/m^3 dose group, both sexes also exhibited epithelial hyperplasia of the ventral (3/5 males, 4/5 females) and ventrolateral (1/5 male, 1/5 female) regions of the larynx.

b) Neoplastic - No neoplastic lesions were produced in this study.

III. DISCUSSION

A. Investigator's Conclusions

Exposure of rats in the 38 and 67 mg/m^3 groups was terminated after 3 days of exposure because of the severity of the toxic reactions. Reactions included marked bodyweight losses, noisy respiration, gasping, lung congestion, and gaseous distension and minimal contents of the gastrointestinal tract. Exposure at 4 and 12 mg/m^3 resulted in treatment related effects including necrosis of the underlying cartilage in the larynx epithelial hyperplasia in the ventral region and squamous metaplasia in the ventrolateral regions. No NOEL was established.

B. Reviewer's Discussion

In the 80 mg/m^3 dose groups, four animals died during exposure, and both sexes exhibited clinical signs of toxicity during and

after exposure. There were marked body weight losses, and reduced food and water consumption. Rats that died exhibited high incidences of lung congestion, and all rats in this group had gaseous distention and minimal contents of the gastrointestinal tract. In the 40 mg/m^3 dose group, one animal died, and both sexes exhibited clinical signs of toxicity during and after exposure. There was significantly reduced bodyweight gain, and reduced food and water consumption in males. For humane reasons, all surviving rats in the 80 and 40 mg/m^3 dose groups were sacrificed after the third exposure.

In the group exposed at 12 mg/m^3, clinical signs of toxicity were observed during and after exposure. Weight gain was decreased significantly in both sexes. After 2 weeks of exposure, males had increased liver weights; both sexes exhibited high incidences of gaseous distention and minimal contents of the cecum; and both sexes exhibited histolopathologic lesions of the respiratory system (epithelial hyperplasia of the ventral region of the larynx, squamous metaplasia in the ventrolateral region of the larynx accompanied by necrosis of the underlying cartilage). In the group exposed at 4 mg/m^3, both sexes exhibited the same histopathologic lesions described above, but clinical signs were absent.

This study was designed and executed to document the LOEL and NOEL of the test material. The LOEL was 4 mg/m^3, and that no NOEL was established. It is noted that a NOEL of 1 mg/m^3 was established in the 5-day inhalation study (MRID # 43491813), based on the same toxicological endpoint (epithelial hyperplasia of the larynx).

IV. STUDY DEFICIENCIES

This study provides supplemental information, but was not conducted according to Subdivision F guidelines for inhalation studies.

The time to equilibrium (Item B4 above) could not be clearly stated.

DATA EVALUATION RECORD

OMACIDE

Study Type: N/A; 5-Day Repeat Dose Inhalation Toxicity Study in Rats

Work Assignment No. 1-8A (MRID 43491813)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer: Mike Norvell, Ph.D.	Signature: Mike Norvell Date: 3/12/96
Secondary Reviewer William McLellan, Ph.D.	Signature: W. L. McLellan Date: 3/12/96
Project Manager William Spangler, Ph.D.	Signature: William J. Spangler Date: 03/12/96
Quality Assurance: Reto Engler, Ph.D.	Signature: Reto Engler Date: 3/12/96

Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

50

EPA Reviewer: T. McMahon, Ph.D. _____, Date 8/7/96
Review Section I, Toxicology Branch II (7509C)
EPA Secondary Reviewer: Y. Ioannou, Ph.D. _____, Date 8/8/96
Review Section I, Toxicology Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Five-Day Inhalation Toxicity - Rat
OPPTS Number: None OPP Guideline Number: None

DP BARCODE: D212164 SUBMISSION CODE: S481260
P.C. CODE: 107801 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Omacide (97-98% ai)

SYNONYMS: IPBC; 3-iodo-2-propynl butylcarbamate

CITATION: Kenny, T.J. 1994. Omacide IPBC. 5-Day Repeat Dose Inhalation Toxicity Study in Rats. Huntingdon Research Centre Ltd., Huntingdon PE18 6ES, England. Laboratory Project Number TXC 8/942212. November 9, 1994. MRID 43491813. Unpublished.

SPONSOR: Olin Corporation, 91 Shelton Avenue, PO Box 30-9643, New Haven, CT 06511

EXECUTIVE SUMMARY:

In an inhalation toxicity study (MRID 43491813), omacide (3-iodo-2-propynl butylcarbamate; >97% ai) was administered to 5 rats/sex/dose by whole-body exposure at nominal concentrations of 0, 0.4, 1.0, 4.0 mg/m^3 (measured concentrations of 0, 0.3, 1.0 and 3.8 mg/m^3, respectively; 0, 0.0003, 0.001, or 0.0038 mg/L, respectively) for 6 hours per day on 5 consecutive days.

Male and female rats in the 4.0 mg/m^3 dose group had histopathologic lesions of the larynx (epithelial hyperplasia in the ventral region and hyperplasia or squamous metaplasia in the ventrolateral regions, with necrosis of the underlying cartilage); in addition, males exhibited a slight reduced body weight gain. Male and female rats in the 0.001 mg/L dose group had the same larynx lesions reported in the 4.0 mg/m^3 dose rats. No adverse effects were seen in the 0.4 mg/m^3 exposure level rats. **The LOEL is 0.001 mg/L (1.0 mg/m^3), based on the presence histopathologic lesions of the larynx. The NOEL is 0.0003 mg/L (0.3 mg/m^3).**

This toxicity study provided **supplemental** data, but was not conducted according to Subdivision F guidelines. It can be used as a pilot range-finding study.

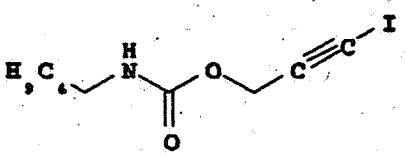
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data

Confidentiality statemets were provided. Flagging statements were not provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Omacide
   Description: White powder
   Lot/Batch #: 2DR-293-TSI
   Purity: >97% ai
   Stability of compound: Expiration date of sample reported to be September 1994
   CAS #: Not provided
   Structure:



2. Vehicle and/or positive control: None

3. Test animals: Species: Rat
   Strain: Sprague Dawley CD
   Age and weight at study initiation: Approximately 6 weeks old; males 249-293 g, females 173-216 g
   Source: Charles River (UK) Limited, Manston Road, Kent, England
   Housing: Housed in groups of 5 in suspended stainless steel cages with mesh tops and bottoms. Exposures took place in the same room.
   Diet: SDS Rat and Mouse #1 SQC Modified Maintenance Diet (Special diet services, Witham Essex, UK)
   Water: municipal tap water, ad libitum
   Environmental conditions:
   Temperature: 19.5-22.0 C
   Relative Humidity: 47-57%
   Air changes: Data not provided
   Photoperiod: 12 hours light/dark cycle
   Acclimation period: 1 day

B. STUDY DESIGN

1. In life dates - Start: 8/26/93 End: 9/9/93 Note: The test substance expiration date was stated as 9/93.

2. Animal assignment

Animals were weighed and assigned by a computer program to obtain approximately equalized group mean body weights to the test groups (Table 1).

TABLE 1. STUDY DESIGN.

Exposure Level	Target Conc. (mg/m^3)	Animals Assigned	
		Males	Females
Control	0	5	5
Low (LCT)	0.4	5	5
Mid (MCT)	1.0	5	5
High (HCT)	4.0	5	5

3. Generation of the test atmosphere and description of the chamber

A Wright dust generator was used to suspend dust in a stream of dry air. The dust laden air was passed through a narrow bore jet into an exhaust nozzle with a baffle and into an elutriator/expansion chamber where it was mixed with air before entering the exposure chamber. Exposure chambers (stainless steel and glass) with an internal volume of approximately 0.75 m^3 were fitted with flow meters and 6 sampling ports. A 3-inch tubular perforated exhaust plenum was situated at the bottom of the chamber and connected to a drainage system.

4. Time to equilibrium

The time to equilibrium was not clearly stated in the report, but duration of exposure to test material was 6 hours with a system air flow of 150 L/min per exposure period (Appendix 3a, page 48 of the report).

5. Analytical Chemistry

The test material was analyzed by HPLC (Appendix 2, pages 45-46 of the report).

Test atmosphere concentration: The chamber nominal concentrations were calculated from the amount of test substance over the exposure periods and the total air flow through the chambers during those periods. At a minimum of approximately 1,3 and 5 hours during each 6-hour exposure period (twice for the control exposures), samples of test atmosphere were withdrawn at 10 L/min through a glass fiber filter supported in an open-faced filter holder. Filters were weighed before and after sampling and then extracted with appropriate solvents for subsequent chemical analysis. Results are summarized as follows:

53

TABLE 2. EXPOSURE PARAMETERS.

Target Conc. (mg/m^3)	Analyzed Conc. (mg/m^3)	Particle Size (%) <6um Diameter
0	0	0
0.4	0.3	66.9
1.0	1.0	72.3
4.0	3.8	70.2

Particle size determination: Chamber air was withdrawn through a Marple 296 cascade impactor at a rate of 2 liters/min. Various impactor stages were used to fractionate the aerosol particulates according to their average aerodynamic equivalent particle diameter. The deposited test article was washed off each stage and analyzed by HPLC according to methods described in Appendix 2 (page 46) of the report. Data in Appendix 5 (page 52 of the report) indicate that the test aerosols did not contain a normal distribution of particle sizes. At least 2 populations of aerosols were generated; one having a mean aerodynamic diameter of approximately 3.5 um and the other with a mean aerodynamic diameter of less than 0.5 um. For this reason, the particle size determination data were summarized as percent of particles with a size less than 6 um diameter.

6. Statistics

For all parameters, the analyses were carried out separately for males and females using the individual animal as the experimental unit. Statistical analyses were not performed for food and water consumption because of the housing (1 cage/sex/group). Bodyweight data were analyzed using weight gains. Bartlett's test was used to test for homogeneity of variance between treatments. If significant heterogeneity was found, a logarithmic transformation was tried to see if a more stable variance structure could be obtained. If no significant heterogeneity was found (or if a satisfactory transformation was found), a one-way analysis of variance was carried out. If significant heterogeneity of variance was detected, and could not be removed by a transformation, the Kruskal-Wallis analysis of ranks was used. Except for pre-exposure data, analysis of variance was followed by Student's 't' and William's tests for dose related responses. The reviewer has no objections to the statistical procedures used in evaluating the data generated in this study.

54

C. METHODS

1. Observations

Animals were inspected twice daily for signs of toxicity and mortality. During exposure, clinical signs were recorded as a group response if all or a majority of visible animals appeared to be responding similarly, or as an individual where one rat was affected.

2. Body weight

Animals were weighed daily throughout the study, beginning one week prior to the first exposure.

3. Food consumption

Food consumption for each cage of animals was recorded daily and mean daily diet consumption was calculated.

4. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subject to gross pathological examination and the following tissues were collected. Histological examination was performed on 4 micron thick sections of the larynx from all rats using H & E stain.

larynx
liver
lung (all lobes and mainstem bronchi)
nasal passages (head for rostral and caudal nasal cavities)

II. RESULTS

A. Observations

1. Mortality - No animals died during the study.

2. Clinical Signs - No significant treatment-related indications of toxicity were observed.

B. Body weight and weight gain

In the 4.0 mg/m^3 dose group, males exhibited a slight, but not statistically significant reduced body weight gain.

C. Food consumption

No significant treatment-related effects on food consumption

55

were reported in this study.

D. Sacrifice and pathology

1. Gross pathology - No significant treatment-related gross pathological lesions were reported in this study.

2. Microscopic pathology - Males and females in the 1.0 and 4.0 mg/m^3 dose groups exhibited epithelial hyperplasia in the ventral region and hyperplasia or squamous metaplasia in the ventrolateral regions of the larynx, with necrosis of the underlying cartilage.

III. DISCUSSION

A. Investigator's Conclusions

The study author concluded that the NOEL was 0.3 mg/m^3, based on epithelial hyperplasia in the ventral region and hyperplasia or squamous metaplasia in the ventrolateral regions with necrosis of the underlying cartilage in rats exposed at nominal rates of 1.0 or 4.0 mg/m^3. The study author did not believe that the slight reduction in bodyweight observed in male rats in the 4.0 mg/m^3 treatment group was treatment-related.

B. Reviewer's Discussion

This study was designed and executed to document the LOEL and NOEL of the test material, and for these purposes is adequate.

IV. STUDY DEFICIENCIES

This study is classified as **supplementary** and does not satisfy Subdivision F guidelines for inhalation studies. It can be used as a pilot range-finding study.

The time to equilibrium (Item B4 above) could not be clearly stated. This minor deficiency did not adversely affect the quality of the study.

55A

DATA EVALUATION RECORD

(12072

OMACIDE

Study Type: 82-1(a); Subchronic Oral Toxicity

Dynamac Study No. 1-08E (MRID 43530202)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer
Joan Harlin, M.S.

Signature: Joan L. Harlin
Date: 4/11/96

Secondary Reviewer
Kathi Ferguson, Ph.D.

Signature: Kathleen Ferguson
Date: 4/11/96

Project Manager
William Spangler, Ph.D.

Signature: William J. Spangler
Date: 4/15/96

Quality Assurance
Reto Engler, Ph.D.

Signature: Reto Engler
Date: 4/15/96

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: T. McMahon, Ph.D. _____, Date 8/7/96
Review Section I, Toxicology Branch II (7509C)
EPA Secondary Reviewer: J. Rowland, Ph.D. _____, Date 8/8/96
Review Section I, Toxicology Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity [gavage] - rats
OPPTS Number: 870.3100 OPP Guideline Number: §82-1a

DP BARCODE: D212151 SUBMISSION CODE: None
P.C. CODE: 107801 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Omacide (IPBC, 97-98% ai)

SYNONYMS: 3-iodopropynylbutylcarbamate

CITATION: Twomey, K. (1994) Omacide (IPBC) 13 week oral (gavage) toxicity study in the rat. Toxicol Laboratories Limited, Herefordshire, England. Laboratory Project ID OLA/24/C. August 16, 1994. MRID 43530202. Unpublished.

SPONSOR: Olin Corporation; 91 Shelton Avenue; P.O. Box 30-9643; New Haven; CT 06511.

EXECUTIVE SUMMARY:

In a subchronic toxicity study (MRID 43530202), Omacide (IPBC; 97-98% ai) was administered to 15 albino rats/sex/dose by gavage at dose levels of 25, 75, or 200 mg/kg/day for 13 weeks.

All treatment groups exhibited excess post-dose salivation; the frequency and severity were concentration-related. Abnormalities in liver function and/or pathology were also observed in all treatment groups. Blood cholinesterase was decreased by 11% in female rats at 75 mg/kg/day and by 17% at 200 mg/kg/day; similar decreases were not observed in the male treatment groups. Two males in the 25 mg/kg/day group exhibited dark livers. Increased incidence of abnormal shape of liver was observed in male rats at all dose levels, and in female rats at 75 and 200 mg/kg/day. Centrilobular hepatocyte hypertrophy was observed in 1/15 males in the 25 mg/kg/day treatment group; 4/15 males in the 75 mg/kg/day treatment group; and 15/15 males and 3/15 females in the 200 mg/kg/day group. Absolute liver weight in the 75 mg/kg/day females was increased 13% vs. controls, and at 200 mg/kg/day, liver weight was increased 29-32% vs. control in male and female rats. Increased relative liver weights were also observed in both sexes at 75 mg/kg/day (12-17% higher) and 200 mg/kg/day (39-41% higher) vs. controls. Also, increased relative kidney weights were noted in the 75 mg/kg/day males and in both sexes from the 200 mg/kg/day groups; no associated macroscopic or microscopic alterations were observed.

Hyperkeratosis and squamous epithelial hyperplasia of the nonglandular region of the stomach were observed in all treatment groups, and stomach ulceration and chronic inflammation were observed in the male and female 200 mg/kg/day treatment groups. This effect was most likely due to the irritancy of the test substance. No treatment-related changes were observed in the clinical appearance, body weight, food consumption or ophthalmology parameters for any of the treatment groups. **The LOEL is 25 mg/kg/day for males and females, based on excessive post-dose salivation in both sexes, abnormal shape of livers in males, and hyperkeratosis and squamous epithelial hyperplasia of the nonglandular region of the stomach in males and females. The NOEL is <25 mg/kg/day for both sexes.**

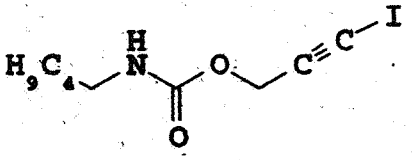
This subchronic toxicity study is graded as **acceptable**, and satisfies the guideline requirement for a subchronic oral toxicity study (§82-1a) in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Omacide
   Description: Off-white crystalline solid
   Lot/Batch #: 2DR-293-TSI
   Purity: 97-98% ai
   Stability of compound: Not provided
   CAS #: Not provided
   Structure:



2. Vehicle and/or positive control: 1% (w/v) aqueous methylcellulose

3. Test animals: Species: Rat
   Strain: CrL:CD(SD)BR (VAF plus)
   Age and weight at study initiation: Approximately 39 days of age; body weights 138-179 g for males, 119-160 g for females

Source: Charles River (UK) Limited, Margate, England
Housing: Groups of five same-sex rats in grid-bottomed stainless steel cages suspended over paper-lined excreta trays
Diet: SQC Rat and Mouse Maintenance Diet No. 1, Expanded (Special Diets Services Limited, Witham, England), ad libitum
Water: tap water in polypropylene bottles, ad libitum
Environmental conditions:
Temperature: 19-22 C
Humidity: 32-68%
Air changes: 16 per hour
Photoperiod: 12-hour light/dark cycle
Acclimation period: 11 days

B. STUDY DESIGN:

1. In life dates - Start: 7/20/93 End: 10/21/93

2. Animal assignment

Sixty rats of each sex were selected for use on the basis of body weight. The selected rats were randomly assigned to the test groups in Table 1 using a stratified body weight procedure.

TABLE 1: STUDY DESIGN

Test Group	Dose to Animal (mg/kg/day)	Animals Assigned	
		Male	Female
1	0[a]	16	15
2	25	15	15
3	75	15	15
4	200	15	15

[a] Controls received 1% (w/v) aqueous methylcellulose by gavage.

3. Dose selection rationale

Dose selection was based on the results of a range-finding study (MRID 43491812) in which Omacide was administered by gavage to male and female rats at concentrations of 50, 125 or 200 mg/kg/day once daily for 14 consecutive days. No treatment-related effects were observed in rats from the

59

50 mg/kg/day treatment groups. In the 125 and 200 mg/kg/day treatment groups, post-dose salivation and, at necropsy, thickening and/or reddening of the glandular mucosa of the stomach were attributed to the irritant properties of the test substance. Both sexes from the 200 mg/kg/day treatment groups had increased relative liver weights. For the 13-week subchronic oral toxicity study, the study author recommended a low dose of 25 mg/kg/day and a high dose level of 200 mg/kg/day, based on the longer dosing period and the known irritant properties of Omacide.

4. Formulation preparation and analysis

The test substance was formulated as a suspension in the vehicle (1% (w/v) aqueous methylcellulose). Separate formulations were prepared fresh each week for each dose level by suspending the required weight of the test substance in the appropriate volume of vehicle. Each formulation was divided into seven aliquots that were stored at 4 C in the dark until use.

Bulk treatment solutions were prepared by diluting 1.0 and 100 mg/mL of the test substance in 1% (w/v) aqueous methylcellulose. Three samples were collected from the top, middle, and bottom of the bulk preparations, and analyzed for homogeneity. The bulk treatment solutions were stored at 4 C for up to 8 days. Samples were analyzed for stability on days 1, 2, 4, and 8. Additional bulk solutions of the test substance were prepared at concentrations of 2.5, 7.5, and 20.0 mg/mL at weeks 0 and 13 of the study, and analyzed to determine the concentration of the test substance in solution.

60

Results (Appendix 11, pages 247-254):
Homogeneity Analysis (Table 2, page 252 of report):

Day 0 (6/28/93)

Concentration (mg/mL)	Homogeneity Level	Amount (mg/mL)	Recovery (%)
1.0	Top	0.9913; 1.024	99-102
	Middle	0.9464; 1.032	95-103
	Bottom	0.9718; 0.9818	97-98
	Mean	0.9900	99
100	Top	90.0; 103.5	90-104
	Middle	104.9; 110.1	105-110
	Bottom	101.0; 104.9	101-105
	Mean	101.9	102

Stability Analysis (duplicate samples):
1 mg/mL: 0 day, 0.9900 mg/mL (99% recovery)
1 day, 1.018; 1.071 mg/mL (102-107% recovery)
2 day, 0.9126; 0.9293 mg/mL (91-93% recovery)
4 day, 0.9935; 1.022 mg/mL (99-102% recovery)
8 day, 0.9867; 1.012 mg/mL (99-101% recovery)
100 mg/mL: 0 day, 101.9 mg/mL (102% recovery)
1 day, 101.6; 105.8 mg/mL (102-106% recovery)
2 day, 97.38; 97.54 mg/mL (97-98% recovery)
4 day, 105.1; 105.4 mg/mL (105% recovery)
8 day, 105.5; 109.6 mg/mL (106-110% recovery)

Concentration Analysis:
Week 0 analysis:
2.5 mg/mL: 2.312; 2.402 mg/mL (92-96% recovery)
7.5 mg/mL: 6.875; 6.975 mg/mL (92-93% recovery)
20.0 mg/mL: 19.45; 20.74 mg/mL (97-104% recovery)
Week 13 analysis:
2.5 mg/mL: 2.264; 2.370 mg/mL (91-95% recovery)
7.5 mg/mL: 7.094; 7.723 mg/mL (95-103% recovery)
20.0 mg/mL: 18.56; 19.19 mg/mL (93-96% recovery)

The analytical data indicated that the mixing procedure was adequate and that the variance between recovery and actual dosage to the animals was acceptable.

61

5. Statistics

Body weight, hematological, and organ weight data were evaluated by analysis of variance. If an intergroup difference at the 5% level occurred, the data were analyzed using pairwise t-tests between the control and treated groups. Hematological and biochemistry data were analyzed using the Kruskal-Wallis one way analysis of variance by ranks test at the 5% significance level. If significance was achieved, the control and treated groups were compared using Dunn's multiple comparison technique.

C. METHODS:

1. Observations

All test animals were inspected daily for changes in physical condition or behavior. All animals were observed twice daily for mortality or signs of morbidity.

2. Body weight

All test animals were weighed on the first day of dosing, weekly throughout the study period, and at necropsy.

3. Food consumption and compound intake

Food consumed within each cage of animals was recorded weekly throughout the treatment period and reported as g/animal/week.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were performed on all test animals prior to study initiation, and on all test animals in the control and 200 mg/kg/day test groups during week 13, using both a direct and an indirect ophthalmoscope after application of a mydriatic agent to both eyes.

5. Blood

Blood was collected from the ten animals of each sex with the lowest identification numbers in each test group during week 13. Blood samples were obtained by puncture of the lateral tail vein following an overnight fast. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc. (MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines.

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
			Direct bilirubin
	ENZYMES	X	Total serum protein (TP)*
			Triglycerides
X	Alkaline phosphatase (ALK)	X	Serum protein electrophores
X	Cholinesterase (ChE)		Albumin/globulin ratio
	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase (also ALT, SGPT)*		
X	Serum aspartate aminotransferase (also AST, SGOT)*		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines.

c. Brain Chemistry

The brains of five animals per test group were cut in the sagittal plane. The right half of each brain was weighed and stored in Triton X-100 prior to analysis for brain cholinesterase. The left half of each brain was fixed and subjected to microscopic examination.

63

6. Sacrifice and Pathology

All test animals that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed. In addition, the following tissues were dehydrated, wax embedded, cut at a nominal thickness of 5 μm, stained with haematoxylin and eosin, and examined microscopically: (i) all tissues from control and high dose animals; (ii) all gross lesions from all animals; and, (iii) livers, kidneys, lungs, and stomachs from all animals.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	X	Heart*	X	Periph.nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*		
X	Duodenum*	X	Spleen*	X	Pituitary*
X	Jejunum*	X	Thymus*	X	Eyes (optic n.)*
X	Ileum*				
X	Cecum*				
X	Colon*		UROGENITAL		GLANDULAR
X	Rectum*				
XX	Liver*+	XX	Kidneys*+	XX	Adrenal gland*
	Gall bladder*	X	Urinary bladder*		Lacrimal gland[T]
X	Pancreas*	XX	Testes*+	X	Mammary gland[T]
		X	Epididymides	X	Parathyroids*++
		X	Prostate	X	Thyroids*++
	RESPIRATORY	X	Seminal vesicle		
		X	Ovaries*+		OTHER
X	Trachea*	X	Uterus*		
X	Lung*			X	Bone*
	Nose			X	Skeletal muscle*
	Pharynx			X	Skin*
	Larynx			X	All gross lesions and masses*

* Required for subchronic studies based on Subdivision F Guidelines
+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
T = required only when toxicity or target organ

II. **RESULTS**

A. Observations

1. Mortality - One control male died on day 2 of the study, and was replaced. No cause of death was established for this animal. One female each from the 25 and 75 mg/kg/day treatment groups died during or after the scheduled bleeding on study day 88. These deaths were considered unrelated to treatment. No other test animals died during the course of the study.

64

2. Clinical Signs - Excess post-dose salivation was observed in both sexes from all treatment groups; the frequency and severity were concentration-related (Table 2). The severity of salivation was minimal in the 25 mg/kg/day groups and minimal to moderate in the 75 and 200 mg/kg/day groups. No other differences in clinical signs were observed between the treatment and control groups.

TABLE 2. THE INCIDENCE OF EXCESS SALIVATION IN MALE AND FEMALE RATS DURING 13 WEEKS OF TREATMENT.[a]

Test Group (mg/kg/day)	# of Affected Animals and Sex	Frequency (Study Days)	Excess Salivation Severity
0	None	---	---
25	2M, 2F	67-71	Minimal
75	3M, 2F	21-24, 67-71	Minimal to moderate
200	4M, 4F	21-40, 43, 45-74, 77-90, 92-93	Minimal to moderate

[a] Data obtained from Appendix 1, page 33, in the study report.

B. Body weight and weight gain

Body weight gains for the 25 mg/kg/day males were comparable to the controls. Body weight gains for the 75 and 200 mg/kg/day males were 7-8% lower and for the female treatment groups were 4-8% lower than the respective controls; the differences were not statistically significant.

TABLE 3. MEAN BODY WEIGHTS AND BODY WEIGHT GAINS FOR RATS.[a]

Test Group (mg/kg/day)	Mean Body Weight (g)		Total Body Weight Gain	
	WEEK 1	WEEK 14[b]	g	% of Control
MALES				
0	158 ±9.0	538 ±41.5	381	--
25	155 ±9.2	540 ±36.6	385	101
75	156 ±9.4	509 ±46.2	353	93
200	156 ±10.2	506 ±61.3	350	92
FEMALES				
0	136 ±10.3	320 ±44.3	185	--
25	133 ±7.9	308 ±26.0	174	94
75	136 ±8.4	313 ±17.3	177	96
200	134 ±7.6	305 ±31.4	171	92

[a] Data obtained from Table 1, pages 25-26, in the study report.
[b] Body weights were determined at necropsy, which was completed in 3 days during week 14.

C. Food consumption

Food consumption for all treated males and females was within 5% and 3% of the respective control values.

D. Ophthalmoscopic examination -

No treatment-related ocular changes or abnormalities were observed.

66

E. Blood work

1. Hematology - Differences in hematological parameters observed between the control and treatment groups were within normal laboratory ranges for rats at 4-6 months of age (range data provided by the registrant on page 96 of this report), and although statistically significant ($p < 0.05$) compared to the controls, were not considered to be treatment-related. These differences included decreases in red blood cell counts of the 75 and 200 mg/kg/day males and the 25 and 200 mg/kg/day females; decreases in hemoglobin of all male treatment groups and the 200 mg/kg/day females; decreases in the hematocrits (packed cell volume; PCV) of all male treatment groups and the 75 and 200 mg/kg/day female; and decreases in mean corpuscular volume (mean cell volume; MCV) and lymphocyte counts of the 200 mg/kg/day females; increases in mean corpuscular hemoglobin concentration (mean cell hemoglobin concentration; MCHC) in both sexes from all treatment groups; and increases in neutrophil counts in the 200 mg/kg/day females (Appendix A). Most of these changes were within 10% of control values, but the changes in RbC, Hb, PCV, and MCV appeared dose-related in both sexes. No other statistically significant differences were observed between the treated and control groups.

2. Clinical Chemistry - Decreases in blood cholinesterase observed in all female treatment groups were concentration-related, reaching statistical significance ($p < 0.05$) in the 75 mg/kg/day (11% lower) and 200 mg/kg/day (17% lower) groups compared to the controls; corresponding decreases were not observed in the male treatment groups (Table 8.2, page 82 of the report).

   Reduced levels of blood urea nitrogen, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin noted in the treatment groups "were in the opposite direction to those normally associated with toxicological effects and thus were considered to be unrelated to treatment [page 20]." Significant ($p < 0.05$) decreases were observed in blood urea nitrogen (BUN) in the 75 and 200 mg/kg/day females (16 and 19% decrease, respectively), ALP in the 200 mg/kg/day females (19% decrease), ALT in all male treatment groups (16-23% decrease), AST in the 25 mg/kg/day males (14% decrease) and in both sexes from the 200 mg/kg/day groups (18 and 28% decrease for males and females, respectively), and bilirubin in all female treatment groups. Other parameters that were statistically significant ($p < 0.05$) but not considered to be treatment-related were glucose (increased in the 25 mg/kg/day males), gamma glutamyl transferase (GGT; decreased in the 25 and 200 mg/kg/day

67

males), A/G ratio (increased in the 75 mg/kg/day females), inorganic phosphorus (decreased in the 25 mg/kg/day males and increased in the 75 mg/kg/day males), and sodium (increased in the 25 mg/kg/day females). In addition, a significant ($p < 0.05$) and dose-related increase was observed for cholesterol levels in all treated male rats and in the 75 and 200 mg/kg/day female rats that were concentration-related. Chloride levels appeared to be increased in all treated male and female rats, but the increase was not dose-related.

F. Sacrifice and Pathology:

1. Organ weights (Table 3, pages 29-30 of the report)- No treatment-related differences in organ weights were observed for both sexes in the 25 mg/kg/day treatment groups. The 200 mg/kg/day males had a significantly ($p < 0.001$) increased absolute liver weight that was 29% higher than the controls (Table 4). The 75 and 200 mg/kg/day males had significantly ($p < 0.01$ and 0.001, respectively) increased relative liver weights that were 12 and 39% higher, respectively, than the control weight. The 75 and 200 mg/kg/day males had significantly increased relative kidney ($p < 0.05$ and 0.001, respectively), brain ($p < 0.05$), and adrenal gland ($p < 0.05$) weights that were likely due to decreased body weights, since the absolute organ weights were not significantly different from the controls, and there were no associated macroscopic or microscopic alterations (Tables 4 and 5). No other significant differences were observed between the organ weights of the treated and control males.

   The 75 and 200 mg/kg/day females had significantly ($p < 0.05$ and 0.001, respectively) increased absolute liver weights that were 13 and 32% higher, respectively, than the control weight (Table 4). Relative liver weights were significantly ($p < 0.001$) increased in the 75 and 200 mg/kg/day females, and were 17 and 41% higher, respectively, than the control weight. The 200 mg/kg females had a significantly ($p < 0.001$) increased relative kidney weight that was likely due to the decreased body weights, since the absolute kidney weight was not significantly different from the controls, and there were no associated macroscopic or microscopic alterations (Table 4). No other significant differences were observed between organ weights of the treated and control females.

TABLE 4. ABSOLUTE AND RELATIVE LIVER AND KIDNEY WEIGHTS OF MALE AND FEMALE RATS AFTER 13 WEEKS OF TREATMENT.[a]

Test Group (mg/kg/day)	Final Body Weight (g)	Liver Weight		Kidney Weight	
		Absolute (g)	Relative	Absolute (g)	Relative
MALES					
0	534	22.58	4.23	3.61	0.68
25	530	22.90	4.30	3.61	0.68
75	498	23.54	4.72**	3.62	0.73*
200	496	29.15***	5.86***	3.78	0.76***
FEMALES					
0	313	11.83	3.77	2.31	0.75
25	299	11.96	4.01	2.27	0.76
75	302	13.35*	4.42***	2.37	0.79
200	293	15.61***	5.33***	2.43	0.83***

[a] Data obtained from Tables 3 and 4, pages 29-32, in the study report.
* Significantly ($p < 0.05$) different from the control.
** Significantly ($p < 0.01$) different from the control.
*** Significantly ($p < 0.001$) different from the control.

TABLE 5. ABSOLUTE AND RELATIVE BRAIN AND ADRENAL GLAND WEIGHTS OF MALE RATS AFTER 13 WEEKS OF TREATMENT.[a]

Test Group (mg/kg/day)	Mean Body Weight (g)	Brain Weight		Adrenal Gland Weight	
		Absolute (g)	Relative	Absolute (mg)	Relative
0	534	2.18	0.41	55	10.33
25	530	2.20	0.42	54	10.22
75	498	2.20	0.45*	57	11.42*
200	496	2.16	0.44*	57	11.62*

[a] Data obtained from Tables 3 and 4, pages 29-32, in the study report.
* Significantly ($p < 0.05$) different from the control.

69

2. Gross pathology - Abnormality of shape of the liver was observed in 3/15 males at 25 and 75 mg/kg/day, and in 9/15 males at 200 mg/kg/day. In females, abnormality of shape of the liver was observed in increased incidence at 75 and 200 mg/kg/day (Table 6). Both sexes from the 75 and 200 mg/kg/day treatment groups exhibited concentration-related increases in enlarged and/or dark livers and thickened and/or pale regions of the stomach mucosa. No other pathological differences were observed between the 75 and 200 mg/kg/day and control groups.

TABLE 6. MACROSCOPIC FINDINGS IN STOMACHS AND LIVERS OF MALE AND FEMALE RATS AFTER 13 WEEKS OF TREATMENT.[a]

Test Group[b] (mg/kg/day)	STOMACH		LIVER	
	Abnormal Color	Abnormal Shape	Abnormal Color	Abnormal Shape
MALES				
0	2	0	0	0
25	1	0	0	3
75	4	9	0	3
200	10	14	2	9
FEMALES				
0	0	0	0	0
25	0	1	0	0
75	4	8	0	4
200	7	14	0	13

[a] Data obtained from Appendix 9, pages 104-107, in the study report.
[b] 15 animals per test group.

3. Microscopic pathology -

a) Non-neoplastic - All treatment groups exhibited hyperkeratosis and squamous epithelial hyperplasia of the nonglandular region of the stomach (Table 7). In the 200 mg/kg/day groups, 3/15 males and 2/15 females had stomach ulceration, and 5/15 males and 4/15 females had chronic stomach inflammation. The incidence of centrilobular hepatocyte hypertrophy in the livers of males from all treatment groups was concentration-related, and was

70

observed in 3/15 females from the 200 mg/kg/day treatment group.

TABLE 7. MICROSCOPIC FINDINGS IN THE STOMACHS AND LIVERS OF MALE AND FEMALE RATS AFTER 13 WEEKS OF TREATMENT[a].

Test Group (mg/kg/day)	Stomach - Nonglandular Region				Liver
	Ulcer	Hyper-keratosis	Chronic Inflammation	Squamous Epithelial Hyperplasia	Centri-lobular Hepatocyte Hypertrophy
MALES					
0	0	0	0	0	0
25	0	10	0	4	1
75	0	9	0	15	4
200	3	15	5	15	15
FEMALES					
0	0	0	0	0	0
25	0	6	0	10	0
75	0	10	0	15	0
200	2	15	4	15	3

[a] Data obtained from Appendix 9, page 102, in the study report.
[b] 15 animals per test group.

b) Neoplastic - No neoplastic tissue was observed in rats in the treatment or control groups.

III. DISCUSSION

A. Investigator's Conclusions

The study author concluded that minimal post-dose excess salivation was the only treatment-related effect observed in the 25 mg/kg/day males and females. The low incidences of stomach alterations observed in both sexes and liver

71

alterations in males were attributed to "the irritancy of the test article, rather than to a direct effect of treatment" [page 10]. In the 75 and 200 mg/kg/day groups, treatment-related effects were slightly depressed body weights (males only), excess post-dose salivation, increased absolute and/or relative liver weights, and enlarged/dark livers and thickening of the stomach mucosa with associated microscopic changes. The author stated that the hyperkeratosis and squamous epithelial hyperplasia in the nonglandular mucosa of the stomach observed in all treatment groups may have been due to the irritancy of the test substance [page 21]. Other alterations observed that were not considered to be treatment-related were altered hematological parameters that were "within laboratory background range" [page 9], reduced clinical chemistry parameters that were "in the opposite direction to those normally associated with toxicological effects" [page 20], and increased relative kidney weights (75 mg/kg/day males, 200 mg/kg/day males and females) in which "there were no pathological findings to support the increased kidney size" [page 22].

B. Reviewer's Discussion

In male and female rats (15 animals/group) administered Omacide via gavage at 25, 75 or 200 mg/kg/day for 13 weeks, excess post-dose salivation was observed; the incidence was concentration-related. No other treatment-related differences were observed in the clinical signs between the treated and control animals.

One female each from the 25 and 75 mg/kg/day treatment groups died during or after the scheduled bleeding on study day 88.

Body weight gains for the 75 and 200 mg/kg/day males were 7-8% lower and for the 25, 75, and 200 mg/kg/day females were 4-8% lower than the respective control gains; the body weight gains for the 25 mg/kg/day males were comparable to the controls.

No treatment-related effects were observed in the food consumption or ophthalmology for any of the treated groups. Altered hematological parameters (decreased red blood cell counts, hemoglobin concentration, and hematocrit, and increased mean corpuscular hemoglobin concentration) were within normal laboratory ranges, and were not considered to be treatment-related, although statistically significant.

Decreases in blood cholinesterase were observed in all female treatment groups and were dose-related, reaching statistical significance in the 75 mg/kg/day (11% lower) and 200 mg/kg/day (17% lower) groups compared to the controls; corresponding decreases were not observed in the male treatment groups. The report stated (page 20) that these reductions were within 20%,

which is regarded as "the percentage reduction required for biologically significant changes in health for humans." However, the dose-related nature of the inhibition should be noted, although blood cholinesterase is not considered as biologically relevant as plasma cholinesterase. Reduced levels of blood urea nitrogen, ALP, ALT, AST, and bilirubin noted in the treatment groups "were in the opposite direction to those normally associated with toxicological effects and thus were considered to be unrelated to treatment" [page 20].

Absolute liver weight in the 75 mg/kg/day females was increased 13% vs. controls, and at 200 mg/kg/day, liver weight was increased 29-32% vs. control in male and female rats. Increased relative liver weights were also observed in both sexes at 75 mg/kg/day (12-17% higher) and 200 mg/kg/day (39-41% higher) vs. controls.

In male rats, absolute liver weight was increased by 4 and 29% at 75 and 200 mg/kg/day, respectively, and relative liver weight was increased by 12 and 39% at these dose levels, respectively. In female rats, absolute liver weight was increased by 13 and 32% at 75 and 200 mg/kg/day, respectively, and relative liver weight was increased by 17 and 41% at these dose levels, respectively. Relative kidney weights for the 75 and 200 mg/kg/day groups were higher than the controls, but no associated macroscopic or microscopic kidney alterations were observed. No other differences in organ weights were observed between the treated and control groups.

Abnormal shape of the liver was observed at 25 mg/kg/day (3 males), 75 mg/kg/day (3 males, 4 females), and 200 mg/kg/day (9 males, 13 females). Dark livers were observed in 2 males from the 200 mg/kg/day group. Thickening of the stomach mucosa was noted at 75 mg/kg/day (9 males, 8 females) and at 200 mg/kg/day (14 males, 14 females). Pallor of the stomach mucosa was observed at 75 mg/kg/day (4 males, 4 females) and at 200 mg/kg/day (10 males, 7 females). No other gross pathological differences were observed between the treated and control groups.

A concentration-related increase in the incidence of centrilobular hepatocyte hypertrophy was observed in all male treatment groups, and was observed in the 200 mg/kg/day females. Hyperkeratosis in the nonglandular mucosa of the stomach was observed at 25 mg/kg/day (10 males, 6 females), 75 mg/kg/day (9 males, 10 females), and 200 mg/kg/day (all test animals). Squamous epithelial hyperplasia in the nonglandular mucosa of the stomach was observed in the 25 mg/kg/day groups (4 males, 10 females) and 75 and 200 mg/kg/day groups (all test animals/group). The incidences of hyperkeratosis and squamous epithelial hyperplasia of the stomach may have been due to the irritancy of the test

substance, rather than a treatment-related effect. In the 200 mg/kg/day groups, the incidences of stomach ulcerations (3 males, 2 females) and chronic inflammation (5 males, 4 females) may also have been due to the irritant properties of the test substance. No other differences in microscopic alterations were observed between the treated and control groups. No neoplastic tissue was observed in the treated or control groups.

IV. Study deficiencies

The concentrations of the test substance were not confirmed. No explanation was provided as to why concentration analyses were conducted using bulk solutions of the test substance at concentrations of 2.5, 7.5, and 20.0 mg/mL in solution, rather than confirming the test concentrations employed in this subchronic toxicity study (25, 75, and 200 mg/kg; equivalent to 0.025, 0.075, and 0.200 mg/mL). However, the homogeneity and stability data submitted in this study are acceptable (recoveries $\geq$90%).

No significant deficiencies from Subdivision F were noted in this study.

DATA EVALUATION RECORD

OMACIDE

Study Type: 83-3a; Prenatal Developmental Study with Rangefinding - Rats

Work Assignment No. 1-8F/1-8G (MRIDs 43491803/43530204)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
Mary Menetrez, Ph.D.
Signature: Mary Menetrez
Date: 3/2/96

Secondary Reviewer
William McLellan, Ph.D.
Signature: W. L. McLellan
Date: 3/2/96

Project Manager
William Spangler, Ph.D.
Signature: William J. Spangler
Date: 03/02/96

Quality Assurance:
Reto Engler, Ph.D.
Signature: Reto Engler
Date: 3/02/96

Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: T. McMahon, Ph.D. _____, Date 8/7/96
Review Section I, Toxicology Branch II (7509C)
EPA Secondary Reviewer: Y. Ioannou, Ph.D. _____, Date 8/8/96
Review Section I, Toxicology Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Study - Rat
OPPTS Number: 870.3700 OPP Guideline Number: §83-3a

DP BARCODE: D212156 SUBMISSION CODE: 481260
P.C. CODE: 107801 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Omacide (97-98% ai, IPBC)

SYNONYMS: 3-iodo-2-propynl butylcarbamate

CITATION: Twomey, K. (1994) Omacide (IPBC): Oral (gavage) rat developmental toxicity (teratogenicity) study. Toxicol Laboratories Limited, Ledbury, Herefordshire, England. Study Nos. OLA/18/R (range-finding) and OLA/19/R. August, 1994. MRIDs 43491803 (range-finding) and 43530204. Unpublished.

SPONSOR: Olin Corporation, 91 Shelton Avenue, PO Box 30-9643, New Haven, CT

EXECUTIVE SUMMARY:

In a developmental toxicity study (MRID 43530204), omacide (>97% ai) was administered to 24 female CD (Sprague-Dawley) rats per dose by gavage at dose levels of 0, 25, 75, or 250 mg/kg/day from days 6 through 15 of gestation.

Maternal toxicity as evidenced by aggressive behavior, post-dose salivation, decreased mean body weight gain, and decreased food consumption was observed in the mid-dose (75 mg/kg/day) and high-dose (250 mg/kg/day) rats. During the dosing period (days 6-15), the mean body weight gain of high- and mid-dose dams were approximately 75 and 83%, respectively of the controls. At the onset of dosing (days 6-9 of gestation), food consumption by the high-dose group was 71% of the controls ($p < 0.01$) and remained lower (not significant) throughout the dosing period. In the mid-dose group, food consumption was approximately 84% of the controls ($p < 0.01$) from days 6 to 12 of gestation. Thereafter, mean food consumption was similar to the controls. Additionally at necropsy, absolute and relative liver weights were approximately 17-22% greater ($p < 0.01$) in the high-dose dams as compared to the controls. **The maternal LOEL is 75 mg/kg/day, based on decreased mean body weight gain and food consumption. The maternal NOEL is 25 mg/kg/day.**

No evidence of a treatment-related effect on fetal viability was

demonstrated. At 250 mg/kg/day, mean fetal body weight was reduced to approximately 95-96% of the controls (significant in the females at $p < 0.05$) and developmental delays that included a higher frequency of rib defects and incomplete or non-ossification of bones were noted. **The developmental LOEL is 250 mg/kg/day based on reduced body weight and developmental delays. The developmental NOEL is 75 mg/kg/day.**

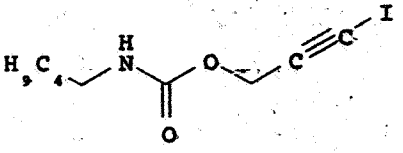
The developmental toxicity study in the rat is classified **acceptable** and does satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3 (a) in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Omacide
   Description: Technical, off-white coarse powder. Formulations in 1% aqueous methylcellulose were stable for 8 days at 4 C.
   Lot/Batch #: 2DR-293-TSI
   Purity: 97-98% ai
   CAS #: 55406-53-6
   Structure:



2. Vehicle:
   Description: 1% Aqueous methylcellulose
   Lot/Batch #: Not specified
   Purity: Not specified

3. Test animals: Species: rat
   Strain: CD (Sprague Dawley)
   Age at mating: 11-12 weeks
   Weight at mating: 223-273 g
   Source: Charles River, U.K. Ltd, England
   Housing: During acclimation, females were housed in grid-bottomed stainless steel cages, 5 per cage. Males were housed in grid-bottomed polypropylene cages. Two females were paired with 1 male in the polypropylene cages during mating. After mating, females were housed in solid-bottomed polypropylene cages with wood sawdust.

77

Diet: SQC Rat and Mouse No. 3 Breeder Diet, ad libitum
Water: Tap water, ad libitum
Environmental conditions:
Temperature: 18-22 C
Humidity: 39-68%
Air changes: 16/hr
Photoperiod: 12 hrs dark/12 hrs light
Acclimation period: 10 days

B. PROCEDURES AND STUDY DESIGN

1. In life dates - Start: 8/30/93 End: 9/20/93

2. Mating: Females were paired on a 2F:1M basis with sexually mature males. Day 0 was designated on the day in which sperm was observed in a vaginal smear.

3. Animal Assignment: Animals were assigned to dose groups as indicated in Table 1. Assignments were randomized and based on body weight.

TABLE 1. ANIMAL ASSIGNMENT.

Test Group	Dose (mg/kg/day)	Number of Females
Control	0	24
Low (LDT)	25	24
Mid (MDT)	75	24
High (HDT)	250	24

4. Dose selection rationale

In a range-finding study (MRID 43491803), omacide was administered orally to groups of 5 female rats each at 0, 50, 150, 200, or 300 mg/kg/day in 1% aqueous methylcellulose. The test solutions were administered at a constant volume of 10 ml/kg on days 6 through 15 of gestation, inclusive. Individual doses were adjusted daily to body weight. Clinical observations, maternal body weight, and food and water consumption data were recorded. On day 20 of gestation, the females were sacrificed and gross necropsy was performed. The uteri and ovaries were examined to determine the status of each conceptus, including the number and distribution of implantations, resorptions, and live or dead fetuses. The liver and gravid uterus weights and the number of corpora lutea were also recorded. Live fetuses were weighed, sexed, and examined

for external abnormalities. In dams dosed at 200 and 300 mg/kg/day the following were observed: reduced maternal body weight gain and food consumption between days 6 and 9 (significantly different from the controls); increased absolute and relative (significantly different) liver weights, and pre- and post-dose salivation. In dams dosed at 150 mg/kg/day, non-significantly reduced maternal body weight gain and food consumption throughout the dosing period were observed, as well as a significant increase in relative liver weight, and pre-and post-dose salivation. There were no maternal effects observed in dams dosed at 50 mg/kg/day.

There were no dose related effects on fetal parameters.

Based upon the results of this range-finding study, 250 mg/kg/day was selected as a high dose for the subsequent full developmental toxicity study in rats. Low- and mid-dose levels chosen were 25 and 75 mg/kg/day, respectively.

5. Dosage preparation and analysis

Test formulations were prepared weekly by mixing appropriate amounts of the test substance with 1% aqueous methylcellulose and stored in the dark in brown glass bottles at 4 C. Prior to the start of the study, homogeneity was tested in samples taken from each of three levels (top, middle, and bottom) of test formulations prepared at low (1.0 mg/ml) and high (100 mg/ml) concentrations in 1% aqueous methylcellulose. Further samples were assayed after 1, 2, 4, and 8 days storage at 4 C to determine stability of the test substance. Concentration analyses of each test formulation were performed twice during the study.

Results:
Homogeneity Analysis: 90-110%
Stability Analysis: 91-107%
Concentration Analysis: 98-104%

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

6. Dosage administration

All doses were administered once daily by gavage, on gestation days 6 through 15, in a volume of 10 ml/kg of body weight. Dosing was based on the most recent body weight determination.

C. OBSERVATIONS

1. Maternal Observations and Evaluations

The animals were checked for mortality or clinical signs daily. Body weights were recorded on days 0, 6 to 15 inclusive, and on day 20 of gestation. Food consumption data were recorded for days 0-6, 6-9, 9-12, 12-15, and 15-20 of gestation. Dams were sacrificed on day 20 of gestation. Thoracic and abdominal cavities were opened, the major organs were examined. Organs or tissues showing macroscopic abnormalities were removed and fixed in formaldehyde. The weight of the whole body, liver, and gravid uterus was recorded. Pregnancy status, number of corpora lutea, and number and distribution of implantation sites were noted. The implantations were classified as early or late resorptions, and dead or live fetuses.

2. Fetal Evaluations

Each fetus was weighed, sexed, and examined for external abnormalities. The neck and thoracic and abdominal cavities of one-half of the fetuses were dissected and examined. Fetuses were eviscerated prior to processing and staining with Alizarin red S for skeletal evaluation. The remaining fetuses were processed in Bouins solution for visceral examination. Fetal findings were classified as variations or abnormalities.

D. DATA ANALYSIS

1. Statistical analyses

Data were subjected to analysis of variance or Kruskal-Wallis test. Where significance was achieved, the data were subjected to Dunnett's t-test ($p < 0.01$ or < 0.05) or Dunn's multiple comparison test ($p < 0.001$, < 0.01, or < 0.05).

2. Indices

Pre-implantation and post-implantation loss indices were calculated from cesarean section records of animals in the study. The pre-implantation loss (%) index was calculated as:

(# corpora lutea - # implantation sites)/# corpora lutea x 100

The post-implantation loss (%) index was calculated as:

(# implantation sites - # live fetuses)/# implantation sites x 100

80

3. Historical control data

Historical control data (July, 1990-September, 1993; 360 mated females) were provided to allow for comparisons with concurrent controls.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations

All dams survived to termination of gestation on day 20. Midway through the dosing period (days 10-16) aggressive behavior and post-dose salivation were observed in all of the mid- and high-dose animals. There were no treatment related clinical observations in the low-dose group.

2. Body Weight

Body weight gain data are summarized in Table 2. During the dosing period (days 6-15), the mean body weight gain for the high and mid-dose dams was approximately 75 and 83%, respectively of the controls; this was significant at $p < 0.01$. The mean body weight gain of females in the low dose treatment group were similar to the controls.

TABLE 2. MATERNAL BODY WEIGHT GAIN (G).[a]

Interval	Dose in mg/kg/day (# of Dams)			
	0 (23)	25 (24)	75 (24)	250 (24)
Pretreatment: Days 0-6	33	32	32	34
Treatment: Days 6-7	4	3	2	-1*
Treatment: Days 6-8	10	11	6*	0*
Treatment: Days 6-9	15	16	11	1*
Treatment: Days 9-12	22	20	17*	20
Treatment: Days 6-15	59	57	49*	44*
Posttreatment: Days 15-20	76	79	83	76

[a] Data obtained from Table 2, page 26, in the study report.
* Significantly different from controls, $p < 0.01$ or < 0.05.

3. Food Consumption

At the onset of dosing (days 6-9 of gestation), food consumption by the high-dose dams was approximately 71% of the controls (significant at $p < 0.01$) and remained lower (not significant) throughout the dosing period. In the mid-dose dams, food consumption was approximately 84% of the controls (significant at $p < 0.01$) from days 6 to 12 of gestation. Thereafter, mean food consumption was similar to the controls. Food consumption by the low-dose group was similar to the controls. The most significant decrease in food consumption (during days 6-15) was accompanied by a decrease in food efficiency during this period (0.88 and 0.84 at the 75 and 250 mg/kg/day dose levels, respectively, vs. 1.0 in control).

4. Gross Pathology

At necropsy, group mean absolute liver weight was

approximately 17-22% greater (significant at p <0.01) in the high-dose dams as compared to the controls, while group mean relative liver weight was approximatelt 22% greater in the high dose group vs control. These findings were judged to be treatment-related. The mean absolute liver weights from the low- and mid-dose dams were similar to the controls; the relative liver weights were greater than the controls, but not statistically different.

An enlarged placenta on two of the six fetuses of one high-dose female and alopecia of the thorax and forelimbs in two of the females from the low-dose group were also observed.

5. Cesarean Section Data

Data are as follows and are summarized in Table 3. The number of corpora lutea, implantations, viable fetuses, placental weights, and the extent of pre- and post-implantation losses were similar between control and treated groups. The number of males in the mid-dose group was significantly (p <0.05) greater than in the controls. However, this was judged unrelated to treatment since it was within the historical range for combined control/inactive treatments. A slight treatment-related reduction in mean fetal weight was observed in both sexes at the high-dose level. The mean high-dose fetal weight was approximately 95-96% of the controls (significant in the females at p <0.05); this finding was below the range of historical controls.

TABLE 3. CESAREAN SECTION OBSERVATIONS.[a]

Observation	Dose (mg/kg/day)			
	0	25	75	250
# Animals Assigned (Mated)	24	24	24	24
# Animals Pregnant Pregnancy Rate (%)	23(96)	24(100)	24(100)	24(100)
Total # Litters	23	24	24	24
Maternal Wastage: # Died (pregnant/nonpregnant)	0	0	0	0
# Aborted	0	0	0	0
# Premature Deliveries	0	0	0	0
Total # Corpora Lutea	398	436	400	448
Corpora Lutea/Dam	17.30	18.17	16.67	18.67
Total # Implantations	376	381	364	386
Implantations/Dam	16.35	15.88	15.17	16.10
Total # Live Fetuses	357	355	349	373
Live Fetuses/Dam	15.5 ± 1.9	14.8 ± 2.9	14.5 ± 3.2	15.5 ± 2.6
Total # Dead Fetuses	0	0	0	0
Total # Resorptions[b]	19	26	15	13
Early	17	23	14	11
Late	2	3	1	2
Resorptions/Dam	0.83	1.08	0.63	0.54
Early	0.74	0.96	0.58	0.46
Late	0.09	0.13	0.04	0.08
Mean Fetal Weight (g) Males	3.75 ± 0.28	3.83 ± 0.29	3.87 ± 0.26	3.60 ± 0.30
Mean Fetal Weight (g) Females	3.55 ± 0.27	3.68 ± 0.25	3.69 ± 0.22	3.37 ± 0.31
Sex Ratio (% Male)	47	53	58	50
Preimplantation Loss (%)	5.1	10.9	8.7	11.7
Postimplantation Loss (%)	5.0	7.0	5.2	3.9

[a] Data obtained from Tables 5 and 6, pages 29-30, and Appendix 7, pages 60-63, in the study report.
[b] No total resorptions were reported.

B. DEVELOPMENTAL TOXICITY

Fetal examinations included external observations at necropsy, visceral observations at necropsy and following free-hand serial sectioning, and skeletal findings. The study report classified fetal findings as variations or malformations (major or minor) and provided a summary incidence of the number of fetuses and mean percent affected in each evaluation category. Noteworthy visceral findings (results of necropsy and serial sectioning) and skeletal observations are summarized in Tables 4a and 4b.

1. External and Visceral Examination

A small number of major abnormalities of the heart, lungs, eyes, and head were noted in the control and treated groups and are summarized in Table 4a. Edema, undescended testes, and cleft palate were observed in two fetuses from one control litter; these abnormalities were not detected in the treated groups. Incidences of minor abnormalities detected outside of the concurrent controls are also noted in Table 4a. Due to the low incidences and the lack of any apparent dose response, the external and visceral abnormalities were not attributed to treatment with omacide.

2. Skeletal Examination

Skeletal examination of the fetuses is summarized in Table 4b. A significant ($p < 0.001$) intergroup difference in skeletal fetal abnormalities was noted by the author. A higher frequency of rib defects and incomplete or non-ossification of bones was detected in the high-dose group compared to the controls. The increased frequency of non-ossification of the 5th sternebrae in the high-dose animals was significantly ($p < 0.001$) different from the controls. There was also a parallel significant ($p < 0.001$) decrease in the number of fetuses with normal sternebral ossification in the high-dose group. These findings are consistent with the slight dose-related decreases in mean fetal body weight noted in the high-dose animals. The frequency of vestigial 14th ribs in all treated groups was higher than the concurrent or historical controls; these findings were judged unrelated to treatment.

TABLE 4a. EXTERNAL AND VISCERAL FETAL OBSERVATIONS AT NECROPSY.[a]

Observations	Dose (mg/kg/day)			
	0	25	75	250
#Fetuses(litters) examined	357 (23)	355 (24)	349 (24)	373 (24)
#Fetuses(litters) affected	5 (2)	1 (1)	5 (3)	6 (4)
Head: Domed (mj)[b]	0 (0)	0 (0)	0 (0)	1 (0.3)
Eye: Uni- or bilateral anophthalmia (mj)	0 (0)	0 (0)	0 (0)	1 (0.7)
Brain: Enlarged lateral ventricles (mi)	1 (0.3)	0 (0)	2 (0.6)	2 (0.5)
Transposition of great vessels (mj)	0 (0)	0 (0)	1 (0.2)	0 (0)
Atria: Enlarged, uni- or bilateral (mj)	0 (0)	0 (0)	0 (0)	1 (0.7)
Heart: Incomplete septum (mj)	0 (0)	0 (0)	1 (0.2)	0 (0)
Lung: Single left or right lobe (mj)	2 (0.6)	0 (0)	2 (0.5)	1 (0.7)
Abdominal hemorrhage (mi)	0 (0)	1 (0.3)	1 (0.2)	2 (0.6)
Situs inversus (mj)	1 (0.3)	0 (0)	1 (0.2)	0 (0)

[a] Number of fetuses (mean %); Mean % = sum of % of affected fetuses per litter/number of litters. Data obtained from Tables 7 and 8, pages 31-32, in the study report.

[b] Abnormality categorized as major (mj) or minor (mi) by author.

TABLE 4b. FETAL SKELETAL EXAMINATIONS - SKULL AND VERTEBRAE.[a]

Observations	Dose (mg/kg/day)			
	0	25	75	250
#Fetuses(litters) examined	179 (23)	177 (24)	175 (24)	187 (24)
SKULL				
Palatine: Cleft (mj)[b]	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)
Hyoid: Not ossified (v)	47 (24.6)	30 (15.4)	36 (18.7)	22 (11.6)
Parietal: Incomplete ossification (v)	4 (1.9)	6 (2.8)	1 (0.6)	3 (3.4)
VERTEBRAE				
No. of thoracic: 14 (mi)	0 (0.0)	0 (0.0)	2 (1.2)	2 (1.1)
No. of lumbar: 7 (mi)	5 (2.8)	2 (1.0)	3 (1.6)	8 (4.7)
Thoracic centra, one or more: Not ossified (mi)	1 (0.6)	2 (1.2)	0 (0.0)	2 (1.8)
Thoracic centra, one or more: Bipartite (mi)	3 (1.7)	0 (0.0)	2 (0.9)	1 (0.6)
Thoracic centra, one or more: Asymmetrically ossified (mi)	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.0)
Thoracic centra, one or more: Hemicentric (mi)	0 (0.0)	0 (0.0)	2 (1.0)	0 (0.0)

[a] Number of fetuses (mean %); Mean %=sum of % of affected fetuses per litter/number of litters. Data obtained from Table 9, pages 33-36, and Appendix 12, pages 88-106, in the study report.

[b] Abnormality categorized as major (mj), minor (mi), or variant (v) by author.

* Significantly different from controls ($p < 0.001$).

87

TABLE 4c. FETAL SKELETAL EXAMINATIONS - RIBS, STERNEBRAE, AND LIMBS.[a]

Observations	Dose (mg/kg/day)			
	0	25	75	250
#Fetuses(litters) examined	179 (23)	177 (24)	175 (24)	187 (24)
RIBS, STERNEBRAE, AND LIMBS				
Ribs, Uni- or bilateral, Vestigial 13th (mi)[b]	0 (0.0)	1 (0.5)	1 (0.7)	1 (0.5)
13 normal pairs (usual)	155 (86.6)	139 (79.7)	132 (76.0)	137 (73.5)
Uni- or bilateral, vestigial 14th (v)	21 (12.0)	37 (19.8)	39 (21.7)	45 (23.7)
Uni- or bilateral, cervical ribs (mi)	1 (0.5)	0 (0.0)	1 (0.5)	5 (2.7)
Sternebrae, 5th: Not ossified (v)	40 (21.6)	21 (12.6)	36 (21.5)	79 (44.1)*
6th: Not ossified (v)	8 (4.3)	7 (4.0)	8 (4.5)	12 (8.3)
1st: Incomplete ossification (mi)	2 (1.0)	2 (1.3)	3 (1.8)	3 (2.3)
2nd: Incomplete ossification (mi)	11 (5.7)	7 (3.8)	6 (3.0)	18 (9.3)
3rd: Incomplete ossification (mi)	2 (1.1)	1 (0.7)	2 (1.3)	3 (2.5)
4th: Incomplete ossification (mi)	4 (2.3)	3 (2.0)	3 (2.0)	7 (5.7)
All sternebrae normal (usual)	128 (72.7)	146 (82.2)	130 (73.8)	93 (47.3)*
Metacarpals, one or more: Not ossified (v)	73 (39.2)	68 (38.5)	62 (32.6)	85 (47.7)
Metatarsals, one or more: Not ossified (mi)	2 (1.0)	0 (0.0)	0 (0.0)	2 (1.9)

[a] Number of fetuses (mean %); Mean %=sum of % of affected fetuses per litter/number of litters. Data obtained from Table 9, pages 33-36, and Appendix 12, pages 88-106 in the study report.

[b] Abnormality categorized as major (mj), minor (mi), or variant (v) by author.

* Significantly different from controls ($p < 0.001$).

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS

The study report concluded that oral administration of omacide at 75 and 250 mg/kg/day to pregnant dams during organogenesis was associated with salivation and aggressive behavior, and decreased body weight and food consumption. In the high-dose group, absolute and relative liver weights in the dams were higher than the controls while fetal weight was lower and the fetuses were less well ossified than the controls. Oral administration of omacide at 75 mg/kg/day produced no adverse developmental effects and dosing at 25 mg/kg/day produced no maternal or developmental adverse effects.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY

Following oral administration of the test substance, omacide (97% ai) to pregnant dams on days 6-15 of gestation, maternal toxicity as evidenced by aggressive behavior, post-dose salivation, decreased mean body weight gain, and decreased food consumption was observed in the mid-dose (75 mg/kg/day) and high-dose (250 mg/kg/day) dams. In addition, at necropsy, absolute and relative liver weights were greater in the high-dose dams as compared to the controls. There were no treatment related effects on mortality, clinical signs, body weight gain, food consumption, gross pathology, or intrauterine parameters in the low-dose group.

Maternal LOEL = 75 mg/kg/day
Maternal NOEL = 25 mg/kg/day

2. DEVELOPMENTAL TOXICITY:

a. Deaths/Resorptions: The numbers of resorptions/dam and viable fetuses/dam for the treatment groups were not significantly different from concurrent or historical controls.

b. Altered Growth: A small treatment-related reduction in mean fetal weight was observed in both sexes at the high-dose (250 mg/kg/day) level (significant in the females at $p < 0.05$). The mean fetal weight (male and female average combined) of 3.485 g is 94% of the historical control mean (3.69 g) and below the low range (3.61).

c. Developmental Variations: A significant ($p < 0.001$) intergroup difference in skeletal fetal abnormalities was

noted by the author. The increased frequency of non-ossification of the 5th sternebrae in the high-dose animals was significantly ($p < 0.001$) different from the concurrent controls and greater than detected in the historical controls. There was also a parallel significant ($p < 0.001$) decrease in the number of fetuses with normal sternebral ossification in the high-dose group. In addition, the frequency of vestigial 14th ribs in all treated groups was higher than the concurrent or historical controls; these findings however, were judged unrelated to treatment.

d. Malformations: A small number of abnormalities of the heart, lungs, eyes, and head were noted in the control and treated groups but were not attributed to treatment with omacide.

Developmental LOEL = 250 mg/kg/day
Developmental NOEL = 75 mg/kg/day

IV. STUDY DEFICIENCIES

The developmental toxicity study in the rat is classified acceptable and does satisfy the guideline requirement for a developmental toxicity study [OPPTS 870.3700; §83-3 (a)] in rats.

012072

OMACIDE IPBC

SALMONELLA/ESCHERICHIA COLI

Principal Reviewer: Nancy E. McCarroll
Review Section III, Toxicology Branch
II/HED (7509C)
Signature: Nancy E. McCarroll
Date: 7/3/96

Secondary Reviewer: Timothy F. McMahon, Ph.D.
Review Section I,
Toxicology Branch II/HED (7509C)
Signature:
Date: 7/3/96

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Salmonella typhimurium / Escherichia coli--mammalian microsome mutagenicity assay; OPPTS 870.5100/5265 [§84-2]

DPBARCODE: D212154 SUBMISSION NO.: S481260

PC CODE: 107801 TOX. CHEM. NO.: 501A MRID No.: 43530207

TEST MATERIAL (PURITY): Omacide IPBC (99%)

COMPOSITION/SYNONYM(S): 3-Iodo-2-propynyl butylcarbamate

CITATION: San, R.H.C. and Klug, M.L. (1993). Salmonella/Mammalian-Microsome Plate Incorporation Assay (Ames Test) and Escherichia coli WP2 uvrA Reverse Mutation Assay With a Confirmatory Assay; Microbiological Associates, Inc., Rockville, MD; Study No. TC727.501088; Study Completion Date: March 31, 1993. Unpublished MRID NUMBER: 43530207

SPONSOR: Olin Corp., New Haven, CT

EXECUTIVE SUMMARY: In a microbial mutagenicity assay (MRID No. 43530207), Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 were exposed to 3.3-1000 µg/plate Omacide IPBC (99%) in the presence and absence of S9 activation and E. coli strain WP2 uvrA was exposed to 10-3333 µg/plate +/- S9. Two independent trials were conducted. The S9 fraction was derived from Aroclor 1254 induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

Cytotoxicity for all strains was observed at 1000 µg/plate +/-S9. The nonactivated and S9-activated positive controls induced the expected mutagenic response in the corresponding tester strain. There was, however, no indication of a mutagenic effect at noncytotoxic doses of Omacide IPBC.

The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for microbial gene mutation mutagenicity data.

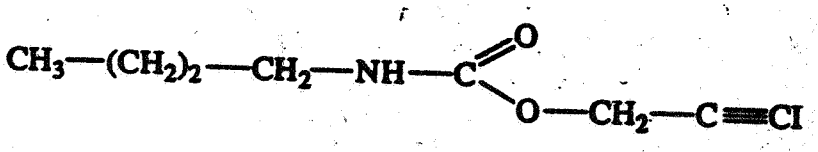
COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Omacide IPBC

Description: White, cream colored crystalline powder
Lot/batch number: Not listed
Purity: 99% (provided by the sponsor)
Receipt date: December 10, 1992
Stability: Not listed.
CAS number: 55406-53-6
Structure:



Solvent used: Dimethyl sulfoxide (DMSO)
Other comments: The test material was stored at 0-6°C, protected from light. Dosing solutions were prepared immediately prior to use and samples were returned to the Sponsor for chemical analysis. The report did not indicate whether analytical determinations were performed.

2. Control Materials:

Negative: None

Solvent/final concentration: DMSO/50 µL per plate

Positive:

Nonactivation:		
Sodium azide	1.0	µg/plate TA1535, TA100
2-Nitrofluorene (2-NF)	1.0	µg/plate TA1538, TA98
9-Aminoacridine (9-AA)	75	µg/plate TA1537
Methyl methanesulfonate (MMS)	1000	µg/plate WP2 uvrA
Activation:		
2-Aminoanthracene (2-AA)	1.0	µg/plate all Salmonella strains
	10	µg/plate E. coli WP2 uvrA

3. Activation: S9 derived from 6.5-7 weeks old male Sprague-Dawley (157-196 g)

x Aroclor 1254	x induced	x rat	x liver
____ phenobarbital	____ noninduced	____ mouse	____ lung
____ none		____ hamster	____ other
____ other		____ other	

The S9 homogenate was prepared by the testing laboratory and assayed prior to use for its ability to convert 2-AA and 7,12-dimethylbenz(a)-anthracene to forms mutagenic to strain TA100.

S9 mix composition:

Component:	Concentration
Phosphate buffer, pH 7.4	100 mM
Glucose-6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10%

4. Test Organism Used: S. typhimurium strains
_____ TA97 __x__ TA98 __x__ TA100 _____ TA102 _____ TA104
__x__ TA1535 __x__ TA1537 __x__ TA1538
list any others: E. coli WP2 uvrA

Test organisms were properly maintained? Yes.
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. Test Compound Concentrations Used:

(a) Preliminary Cytotoxicity assay: Ten doses (6.7, 10, 33, 67, 100, 333, 667, 1000, 3333 and 5,000 µg/plate) were evaluated with strains TA100 and WP2 uvrA in the presence and absence of S9 activation; single plates were used per dose, per strain, per condition.

(b) Mutation assays:

Trial 1: Trial 1 was aborted due to a technical error.

Trial 2: Six doses (3.3, 10, 33, 100, 333 and 1000 µg/plate) were evaluated in triplicate in the presence and absence of S9 activation with all Salmonella tester strains. The six non-activated and S9-activated doses evaluated in the E. coli strain ranged from 10-3333 µg/plate +/-S9. Triplicate plates were used per dose, per strain, per condition.

Trial 3: Doses and conditions were as described for Trial 2.

B. TEST PERFORMANCE:

1. Type of Salmonella Assay: __x__ Standard plate test
_____ Pre-incubation (_____) minutes
_____ "Prival" modification
_____ Spot test
_____ Other (describe)

93

2. Protocol: Similar procedures were used for the preliminary cytotoxicity and the mutation assays. A 100-µL aliquot of the appropriate tester strain at a density of ≈10^9 cells/mL and 50 µL of the appropriate test material dose, solvent, or positive control were mixed with 2.5 mL volumes of molten top agar supplemented with L-histidine and biotin (S. typhimurium strains) or tryptophan (E. coli strain) In the S9-activated tests, 0.5 mL of the S9-cofactor mix was added to 2.0 mL of top agar. The contents of the tubes were mixed, poured over Vogel-Bonner minimal medium E plates and incubated at 37±2°C for ≈48 hours. At the end of incubation, plates were either scored immediately for revertant colonies or were refrigerated and subsequently counted. Means and standard deviations for the mutation tests were determined from the counts of triplicate plates per strain, per dose, per condition. The sterility of the S9 mix and the most concentrated test material dosing solution used in the mutation assays was determined by plating on selective agar.

3. Evaluation Criteria:

(a) Assay validity: The assay was considered acceptable if (1) the appropriate genetic markers were verified for each tester strain, (2) the number of spontaneous revertants for each tester strain was within specified limits, (3) the density of the tester strain cultures was ≥0.3x10^9 cells/mL, and (4) the nonactivated and S9-activated positive controls induced at least a 3-fold increase in the number of revertant colonies compared to the solvent control.

(b) Positive response: The test material was considered positive if it caused a dose-related increase in the mean number of revertants per plate of at least one strain. This increase must be at least 2-fold in strains TA98, TA100 and WP2 uvrA or at least 3-fold in strains TA1535, TA1537 and TA1538 at the peak of the response.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: Ten doses of the test material ranging from 6.7 to 5,000 µg/plate were evaluated with and without S9 activation using strains TA100 and WP2 uvrA. No compound precipitation was apparent at any nonactivated or S9-activated level. Concentrations ≥667 or ≥3333 µg/plate +/-S9 were lethal in strain TA100 or WP2 uvrA, respectively. Cytotoxicity, as indicated by an ≈50% or greater reduction in revertant colonies was also apparent at 333 µg/plate + S9 (TA100) or at 1000 µg/plate +/- S9 (WP2 uvrA).

2. Mutation assays: Due to a technical error, the original assay was aborted. Summarized results from the successfully completed trials are presented in Study Report Tables 27 and 28, pp 43 and 44 (see Attachments). As shown, the data from both assays were in good agreement and indicated that Omacide IPCB was cytotoxic for all strains at 1000 µg/plate +/- S9 but was not mutagenic. The slight increases in revertant colonies of strains TA1535, TA1537 and TA100 noted in the confirmatory trial at 333 µg/plate + S9 were not sufficient to conclude

94

a positive response. These increases were not seen in the initial trial and all values were within the historical background ranges of the reporting laboratory. By contrast, the nonactivated and S9-activated positive controls induced clear mutagenic responses in the appropriate tester strain in both trials.

Based on the overall findings, the study authors concluded that Omacide IPBC was not mutagenic in this microbial gene mutation assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study was properly conducted and we concur with the study authors' conclusion that Omacide IPBC was assayed over an appropriate dose range but failed to induce a genotoxic response. The sensitivity of the test system to detect mutagenesis was adequately demonstrated by the results obtained with the nonactivated and S9-activated positive controls. We conclude, therefore, that the study provided acceptable evidence that Omacide IPBC is negative in this microbial test system.

E. STUDY DEFICIENCIES: None.

95

ATTACHMENT

STUDY REPORT TABLES 27 and 28 PP. 43 AND 44

OMACIDE TOX R 012072

Page_____ is not included in this copy.

Pages 97 through 98 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

X FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

212072

OMACIDE IPBC **GENE MUTATIONS IN CULTURED MAMMALIAN CELLS (84-2)**

Principal Reviewer: Nancy E. McCarroll Signature: Nancy E. McCarroll
Review Section III, Toxicology Branch Date: 7/3/96
II/HED (7509C)
Secondary Reviewer: Timothy F. McMahon, Ph.D. Signature:
Review Section I,
Toxicology Branch II/HED (7509C) Date: 7/3/96

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Mammalian Cells in Culture Gene Mutation Assay in Chinese Hamster Ovary (CHO) Cells; OPPTS 870.5300 [§84-2]

DPBARCODE: D212151 SUBMISSION NO.: S481260

PC CODE: 107801 TOX. CHEM. NO.: 501A MRID No.: 43491805

TEST MATERIAL (PURITY): Omacide IPBC (97.5%)

COMPOSITION/SYNONYM(S): 3-Iodo-2-propynyl butylcarbamate

CITATION: Bigger, C.A.H. and Clarke, J.J (1993). CHO/HGPRT Mutation Assay With Confirmation; Microbiological Associates, Inc., Rockville, MD; Study No. TC727.332001; Study Completion Date: September 1, 1993. Unpublished MRID NUMBER: 43491805

SPONSOR: Olin Corp., New Haven, CT

EXECUTIVE SUMMARY: In an in vitro mammalian cell gene mutation study (MRID No. 43491805), Chinese hamster ovary (CHO) cells were exposed to Omacide IPBC (97.5%) doses of 5-20 µg/mL without S9 activation or 15-100 µg/mL with S9 activation in the initial trial. Concentrations evaluated in the confirmatory trial ranged from 2.5-20 µg/mL -S9 or 15-50 µg/mL +S9. The S9 fraction was derived from Aroclor 1254 induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

Cytotoxicity was apparent at ≥15 µg/mL -S9 and at ≥50 µg/mL +S9. The nonactivated and S9-activated positive controls induced the expected mutagenic response. There was, however, no indication that Omacide IPBC induced a mutagenic effect.

The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for mammalian cell gene mutation mutagenicity data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

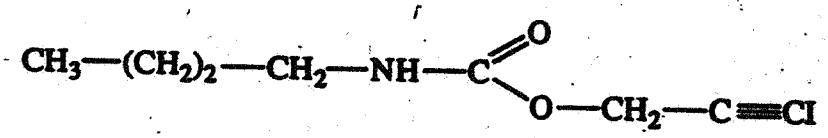
99

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Omacide IPBC

Description: White/cream colored crystalline powder
Lot/batch number: Not listed
Purity: 97.5% (provided by the sponsor)
Receipt date: December 10, 1992
Stability: Not listed.
CAS number: 55406-53-6
Structure:



Solvent used: Dimethyl sulfoxide (DMSO)
Other comments: The test material was stored at 0-6°C, protected from light. The frequency of dosing solution preparation was not reported. Samples of dose formulations made for the mutation studies were returned to the Sponsor for chemical analysis. The report did not indicate whether analytical determinations were performed.

2. Control Materials:

Negative: Ham's F-12 medium supplemented with 5% fetal bovine serum, 1% L-glutamine, and antibiotics

Solvent/final concentration: DMSO/1%

Positive: Nonactivation (concentrations, solvent): Ethyl methanesulfonate (EMS) was prepared in DMSO to yield a final concentration of 0.2 µL/mL.

Activation (concentrations, solvent): Benzo(a)pyrene (BaP) was prepared in DMSO to yield final concentrations of 4 and 5 µg/mL.

3. Activation: S9 derived from adult male Sprague-Dawley Fischer

x Aroclor 1254	x induced	x rat	x liver
_____ phenobarbital	_____ noninduced	_____ mouse	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ other	

The S9 homogenate (Batch Nos. R462 and R475) was prepared by the performing laboratory. Prior to use the S9 fraction was characterized for its ability to metabolize 2-aminoanthracene and 7,12-dimethylbenz(a)anthracene to mutagenic forms in Salmonella typhimurium TA100.

100

S9 mix composition:

Component	Concentration/mL of Culture Medium
NADP (sodium salt)	4 mM
Glucose 6-phosphate	5 mM
Potassium chloride	30 mM
Magnesium chloride	10 mM
Sodium phosphate buffer, pH 8.0	50 mM
Calcium chloride	10 mM
S9 homogenate	100 µL/mL of cofactor mix

4. Test Cells: Mammalian cells in culture

_____ mouse lymphoma L5178Y cells
__x__ Chinese hamster ovary (CHO) cells
_____ V79 cells (Chinese hamster lung fibroblasts)
_____ other (list):

Properly maintained? Yes.
Periodically checked for mycoplasma contamination? Yes.
Periodically checked for karyotype stability? Not reported.
Periodically "cleansed" against high spontaneous background? Yes.

5. Locus Examined:

_____ thymidine kinase (TK)
selection agent: _____ bromodeoxyuridine (BrdU)
(give concentration) _____ fluorodeoxyuridine (FdU)

__x__ hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)
Selection agent: _____ 8-azaguanine (8-AG)
(give concentration) 10 µM 6-thioguanine (6-TG)

_____ Na^+/K^+ATPase
Selection agent: _____ ouabain
(give concentration)

_____ other (locus and/or selection agent; give details):

6. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: Nine doses (0.5, 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg/mL) were evaluated with and without S9 activation.

101

(b) Mutation assays:

Initial Trial: 5, 7.5, 10, 12.5, 15 and 20 μg/mL -S9
15, 25, 50, 60, 75 and 100 μg/mL +S9

Confirmatory Trial: 2.5, 5, 7.5, 10, 12.5, 15 and 20 μg/mL -S9
15, 25, 30, 40 and 50 μg/mL +S9

Note: Owing to contamination, three trials of the S9-activated confirmatory assay were attempted. Only the data from the successfully completed trial were reported.

B. TEST PERFORMANCE:

1. Cell Treatments:

(a) Cells exposed to test compound, negative, solvent and positive controls for:
5 hours (nonactivated) 5 hours (activated)

(b) After washing, cells cultured for 7 to 9 days (expression period) before cell selection.

(c) After expression, cells were plated at a density of $2x10^5$ cells/dish (10 plates/dose) and cultured for 7-10 days in selection medium to determine numbers of mutants. Cells were plated at a density of 100 cells/dish (6 plates/dose) and cultured and for 7-10 days without selection medium to determine cloning efficiency (CE).

2. Statistical Methods: Statistical analysis was not performed.

3. Evaluation Criteria:

Assay validity: The assay was considered valid if the following criteria were met: (1) the CE of the untreated and solvent controls was ≥50%; (2) the spontaneous mutation frequency (MF) in the negative and solvent control groups fell within the range of 0 to 25 mutants/10^6 cells; (3) the positive controls induced MFs that were ≥3-fold higher than the solvent control and exceed the minimum MF (40 mutants/10^6 cells) considered indicative of a positive response.

Positive response: The test material was considered positive in this assay system if at least two test doses induced MFs that exceeded 40 mutants/10^6 cells and the increase was accompanied by a dose response.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assessment: The osmolality and pH of the highest dose prepared for the initial cytotoxicity assessment

102

TABLE 1. Representative Results of the Initial CHO/HGPRT Forward Gene Mutation Assay with Omacide IPBC

Substance	Dose (µg/mL)	S9	Relative % Cloning Efficiency (after treatment)	Total Mutant Colonies	Cloning Efficiency (at selection)	Mutation Frequency/ 10^6 cells[a]
Negative Control						
Culture medium	--	-	111	15	0.98	7.7
	--	+	81	6	0.93	3.2
Solvent Control						
Dimethyl sulfoxide	1%	-	100	14	0.94	7.4
	1%	+	100	46	0.98	23.4
Positive Control						
Ethyl methanesulfonate	0.2 µL	-	72	258	0.84	170.0
Benzo(a)pyrene[b]	4.0	+	17	113	0.77	73.9
Test Material						
Omacide IPBC	10.0[c]	-	33	49	0.85	28.7
	12.5	-	23	11	1.00	5.5
	15.0[d]	-	8	0	0.66	<1.1[e]
	25.0[c]	+	53	34	0.66	28.5
	50.0	+	13	3	0.76	2.0
	60.0[d]	+	10	0	0.81	<0.6[e]

[a]Mutation Frequency (MF) = $\frac{\text{Total Mutant Colonies}}{\text{Number of Selection Plates (10) x Cloning Efficiency x } 2\text{x}10^5 \text{ cells}} \times 10^6$.

[b]Two levels were assayed, data from the lower dose were selected as representative.

[c]Findings for lower doses (5.0 or 7.5 µg/mL -S9 or 15 µg/mL +S9) did not indicate a mutagenic effect.

[d]Higher doses (20 µg/mL -S9; ≥75 µg/mL +S9) were severely cytotoxic.

[e]Calculated on the basis of <1 mutant colony in the total number of selection plates.

Note: Data were extracted from the Study Report, Tables 2-4 pp. 15-17.

TABLE 2. Representative Results of the Confirmatory CHO/HGPRT Forward Gene Mutation Assay with Omacide IPBC

Substance	Dose (µg/mL)	S9	Relative % Cloning Efficiency (after treatment)	Total Mutant Colonies	Cloning Efficiency (at selection)	Mutation Frequency/ 10^6 cells[a]
Negative Control						
Culture medium	--	-	97	19	0.93	10.2
	--	+	119	0	1.17	<0.4[b]
Solvent Control						
Dimethyl sulfoxide	1%	-	100	34	0.92	18.4
	1%	+	100	0	1.19	<0.4[b]
Positive Control						
Ethyl methanesulfonate	0.2 µL	-	66	442	0.79	281.5
Benzo(a)pyrene[c]	4.0	+	64	207	0.88	117.6
Test Material						
Omacide IPBC	12.5[d]	-	27	0	0.83	<0.6[b]
	15.0	-	10	2	0.84	1.2
	20.0	-	5	0	0.93	<1.8[b]
	25.0[d]	+	47	3	1.01	1.5
	30.0	+	65	8	1.20	3.3
	40.0	+	20	13	1.35	4.8
	50.0	+	8	0	1.18	<0.5[b]

[a]Mutation Frequency (MF) = $\frac{\text{Total Mutant Colonies}}{\text{Number of Selection Plates (10) x Cloning Efficiency x } 2\text{x}10^5 \text{ cells}} \times 10^6$.

[b]Calculated on the basis of <1 mutant colony in the total number of selection plates counted.

[c]Two levels were assayed, data from the lower dose were selected as representative.

[d]Findings for lower doses (2.5, 5.0, 7.5 or 10 µg/mL -S9 or 15 µg/mL +S9) did not indicate a mutagenic effect.

Note: Data were extracted from the Study Report, Tables 5-7 pp. 18-20.

104

OMACIDE IPBC MICRONUCLEUS (84-2)

Principal Reviewer: Nancy E. McCarroll Signature: Nancy E. McCarroll
Review Section III, Toxicology Branch Date: 7/3/96
II/HED (7509C)
Secondary Reviewer: Timothy F. McMahon, Ph.D. Signature:
Review Section I,
Toxicology Branch II/HED (7509C) Date: 7/3/96

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Mouse micronucleus assay; OPPTS 870.5395 [§84-2]

DPBARCODE: D212153 SUBMISSION NO.: S481260

PC CODE: 107801 TOX. CHEM. NO.: 501A MRID No.: 43530206

TEST MATERIAL (PURITY): Omacide IPBC (97.5%)

COMPOSITION/SYNONYM(S): 3-Iodo-2-propynyl butylcarbamate

CITATION: Putman, D.L. and Young, R.R. (1993). Micronucleus Cytogenetic Assay in Mice; Microbiological Associates, Inc., Bethesda/ Rockville, MD; Study No. TC727.122; Study Completion Date: May 10, 1993. Unpublished MRID NUMBER: 43530206

SPONSOR: Olin Corp., New Haven, CT

EXECUTIVE SUMMARY: In a mouse micronucleus assay (MRID No: 43530206), groups of five male and five female ICR mice received single intraperitoneal injections of 28, 55 or 110 mg/kg Omacide IPBC (97.5%) prepared in corn oil. Animals were sacrificed at 24, 48 or 72 hours postexposure and bone marrow cells were examined for the incidence of micronucleated polychromatic erythrocytes (MPEs).

Overt toxicity in high-dose animals included death and lethargy. There was no evidence of a cytotoxic effect on the target tissue. The positive control induced the expected high yield of MPEs in both sexes. There was, however, no evidence that Omacide IPBC induced a clastogenic or aneugenic effect.

The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for in vivo cytogenetic mutagenicity data.

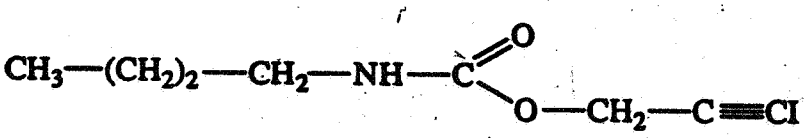
COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Omacide IPBC

Description: White, cream colored crystalline powder
Lot/batch number: 2DR-293-TSI
Purity: 97.5% (provided by the sponsor)
Receipt date: December 10, 1992
Stability: Not listed.
CAS number: 55406-53-6
Structure:



Vehicle used: Corn oil
Other provide information: The test material was stored at 0-6°C, protected from light. Aliquots of the dosing solutions used in the micronucleus assay were returned to the sponsor for chemical analysis; there was, however, no indication in the report that analytical determinations were carried out.

2. Control Materials:

Negative/Route of administration: None

Vehicle/Final concentration/Route of administration: Corn oil was administered by intraperitoneal (IP) injection at a dosing volume of 20 mL/kg.

Positive/Final concentration/Route of administration:
Cyclophosphamide (CP) was dissolved in distilled water at a concentration of 1.5 mg/mL and was administered IP at 30 mg/kg.

3. Test Compound:

Route of administration: IP

Dose levels used:

(a) Pilot study: 1, 10, 100 and 1000 mg/kg (2 ♂/dose)
5000 mg/kg (5 ♂ and 5 ♀)

(b) Toxicity test 1: 150, 300, 600 and 900 mg/kg
(5 ♂ and 5 ♀/dose)

(c) Toxicity test 2: 10, 50, and 100 mg/kg
(5 ♂ and 5 ♀/dose)

106

(d) Micronucleus assay: 28, 55 and 110 mg/kg
(5 ♂ and 5 ♀/dose or vehicle control group/sacrifice time)
(5 ♂ and 5 ♀--positive control group)

Note: An additional group of 5 males and 5 females received the high dose and were used to replace animals that died in the primary micronucleus assay high-dose group.

4. Test Animals:

(a) Species Mouse Strain ICR Age 6-8 weeks

Weight range at randomization:

- Pilot study: 29.5-32.6 g (♂); 23.0-24.6 g (♀)
- Toxicity test 1: 32.8-40.3 g (♂); 22.6-25.6 g (♀)
- Toxicity test 2: 28.9-32.6 g (♂); 23.2-25.9 g (♀)
- Micronucleus assay: 28.3-35.1 g (♂); 21.6-25.3 g (♀)

Source: Harlan Sprague Dawley, Inc., Frederick, MD

(b) No. animals used per dose: See Section A 3 (a - d).

NOTE: All animals were weighed immediately before compound administration; dosing was based on individual body weights.

(c) Properly maintained? Yes.

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

(a) Test compound and vehicle control:
Dosing: x once _____ twice (24 hr apart)
____ other (describe): _____
Sampling (after last dose): _____ 6 hr _____ 12 hr
x 24 hr x 48 hr x 72 hr

(b) Positive control:
Dosing: x once _____ twice (24 hr apart)
____ other (describe): _____
Sampling (after last dose): x 24 hr _____ 48 hr
_____ 72 hr

2. Tissues and Cells Examined:

___x___ bone marrow __N/A__ others (list):
Number of polychromatic erythrocytes (PCEs) examined per animal: __1000__
Number of normochromatic erythrocytes (NCEs, more mature RBCs) examined per animal: 1000 total erythrocytes.

3. Details of Slide Preparation: At 24, 48 and 72 hours after administration of the test material or the vehicle control, the appropriate groups of animals were sacrificed by CO_2 asphyxiation. Sacrifice time for the positive control group was 24 hours. Bone marrow cells were aspirated from both femurs into fetal bovine serum, centrifuged, resuspended in residual supernatant and spread onto slides. Prepared slides were fixed in methanol, stained with May-Gruenwald-Giemsa solution, and coverslipped. Slides were coded prior to scoring.

4. Statistical Methods: The data were evaluated for statistical significance at $p \leq 0.05$ using the Kastenbaum-Bowman tables.

5. Evaluation Criteria:

(a) Assay validity: The study was considered valid if the mean number of micronucleated polychromatic erythrocytes (MPEs) in the negative (vehicle) control did not exceed 0.5%, and the positive control induced a significant ($p \leq 0.05$) increase in MPEs.

(b) Positive response: The test material was considered positive for micronuclei induction if a significant ($p \leq 0.05$) and dose-related increase in MPEs compared to the vehicle control was seen at any sacrifice time.

C. REPORTED RESULTS:

1. Pilot Study: Animals administered the selected doses of the test material were weighed immediately prior to dosing and on days 1 and 3 postdosing. Clinical signs and mortality were monitored immediately after dosing and daily, thereafter, for 7 days. All animals receiving 1000 or 5000 mg/kg died within 1 day of compound administration. Lethargy in male mice at 100 mg/kg was the only other reported sign of compound toxicity in survivors. Animals dosed with either 1 or 10 mg/kg appeared normal throughout the observation period.

2. Toxicity Test 1: Based on the results of the pilot study, a second evaluation of toxicity was undertaken with four doses of the test material (150, 300, 600 and 900 mg/kg) administered IP to groups of five male and five female mice. Body weights, mortality and clinical signs were recorded as described in the pilot study. With the exception of a single female exposed to 300 mg/kg, all animals succumbed to treatment within 2 days of compound administration. Consequently, a further

toxicity assessment was performed with test article doses of 10, 50 and 100 mg/kg.

3. Toxicity Test 2: Results from the additional round of toxicity testing indicated that no deaths or signs of clinical toxicity were evident throughout the observation period. From the overall results of the three preliminary studies, the $LD_{50/7}$, calculated by probit analysis, was estimated to be 137 mg/kg for both sexes. Accordingly, the high dose selected for the micronucleus assay (≈80% of the $LD_{50/7}$) was 110 mg/kg; lower levels selected for study were 55 and 28 mg/kg.

4. Micronucleus Assays:

(a) Animal observations: One male receiving the high dose was found dead on Day 2 and was replaced with a secondary group male. Lethargy and diarrhea were also recorded for the high-dose group. All remaining animals appeared normal throughout the course of study.

(b) Bone marrow analysis: Summarized results from the micronucleus assay conducted with Omacide IPBC administered IP to male and female mice are presented in Study Report Table 2, p 15 (see Attachment). As shown, exposure to the selected dose of the test material did not adversely affect the PCE:NCE ratio. Similarly, there were no appreciable increases in the frequency of MPEs in any treatment group or at any sacrifice time. By contrast, the positive control (30 mg/kg CP) induced a significant ($p \leq 0.05$) genotoxic response in both sexes.

From the overall findings, the study authors concluded that Omacide IPBC was negative in the mouse micronucleus assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We agree with the study authors' assessment that Omacide IPBC was neither clastogenic nor aneugenic in this *in vivo* assay. The evidence of overt compound toxicity in the high-dose group (110 mg/kg) indicates that an appropriate range of test material concentrations was evaluated. Additionally, the sensitivity of the assay system to detect a positive response was adequately demonstrated by the significant ($p \leq 0.05$) results obtained with 30 mg/kg CP. We conclude, therefore, that the study provided acceptable evidence that Omacide IPBC is not genotoxic in this whole animal test system.

E. STUDY DEFICIENCIES: NONE.

109